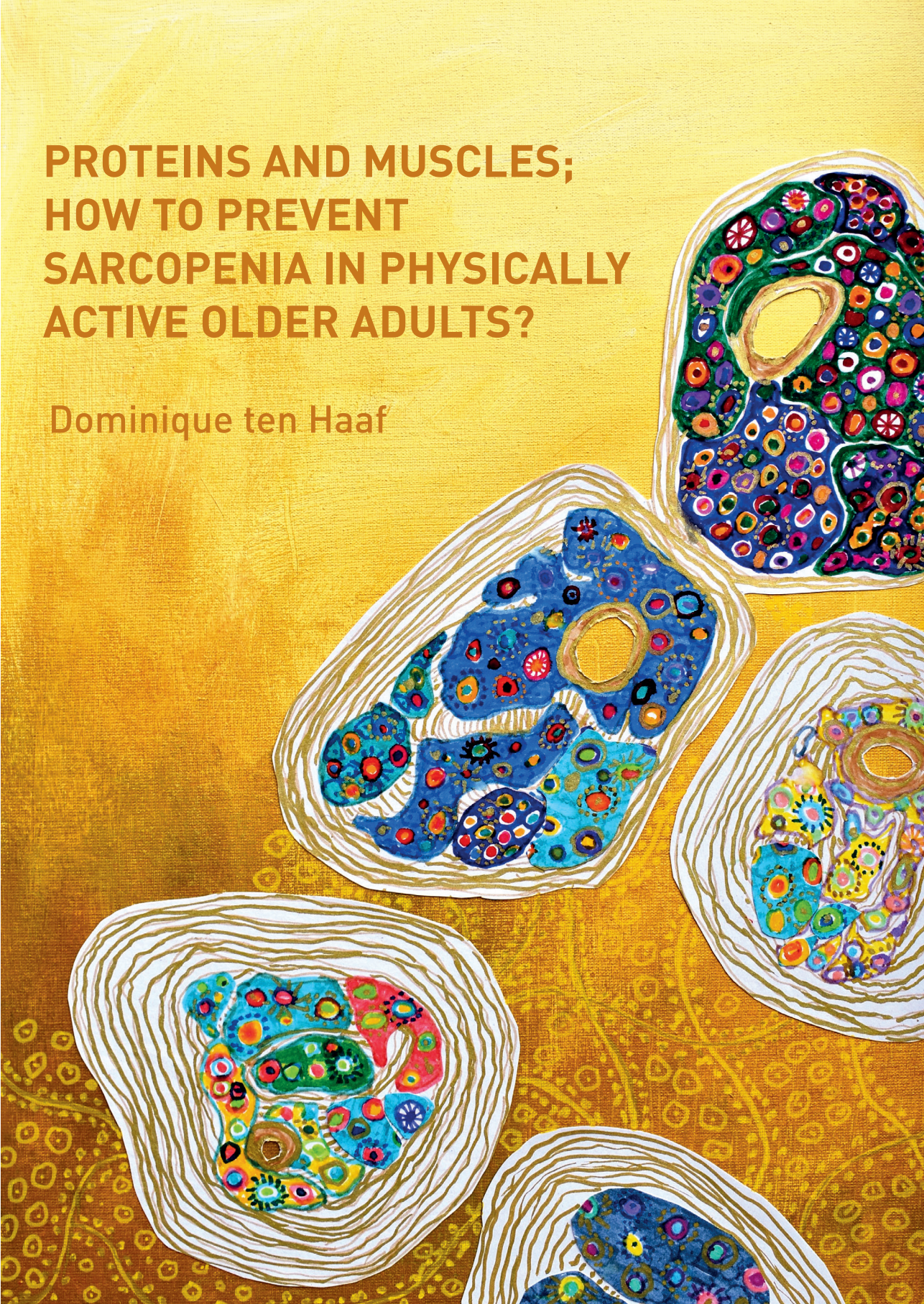
PROTEINS AND MUSCLES; HOW TO PREVENT SARCOPENIA IN PHYSICALLY ACTIVE OLDER ADULTS?

Dominique ten Haaf



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Proteins and muscles; How to prevent sarcopenia in physically active older adults?
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PROTEINS AND MUSCLES; HOW TO PREVENT SARCOPENIA IN PHYSICALLY ACTIVE OLDER ADULTS?

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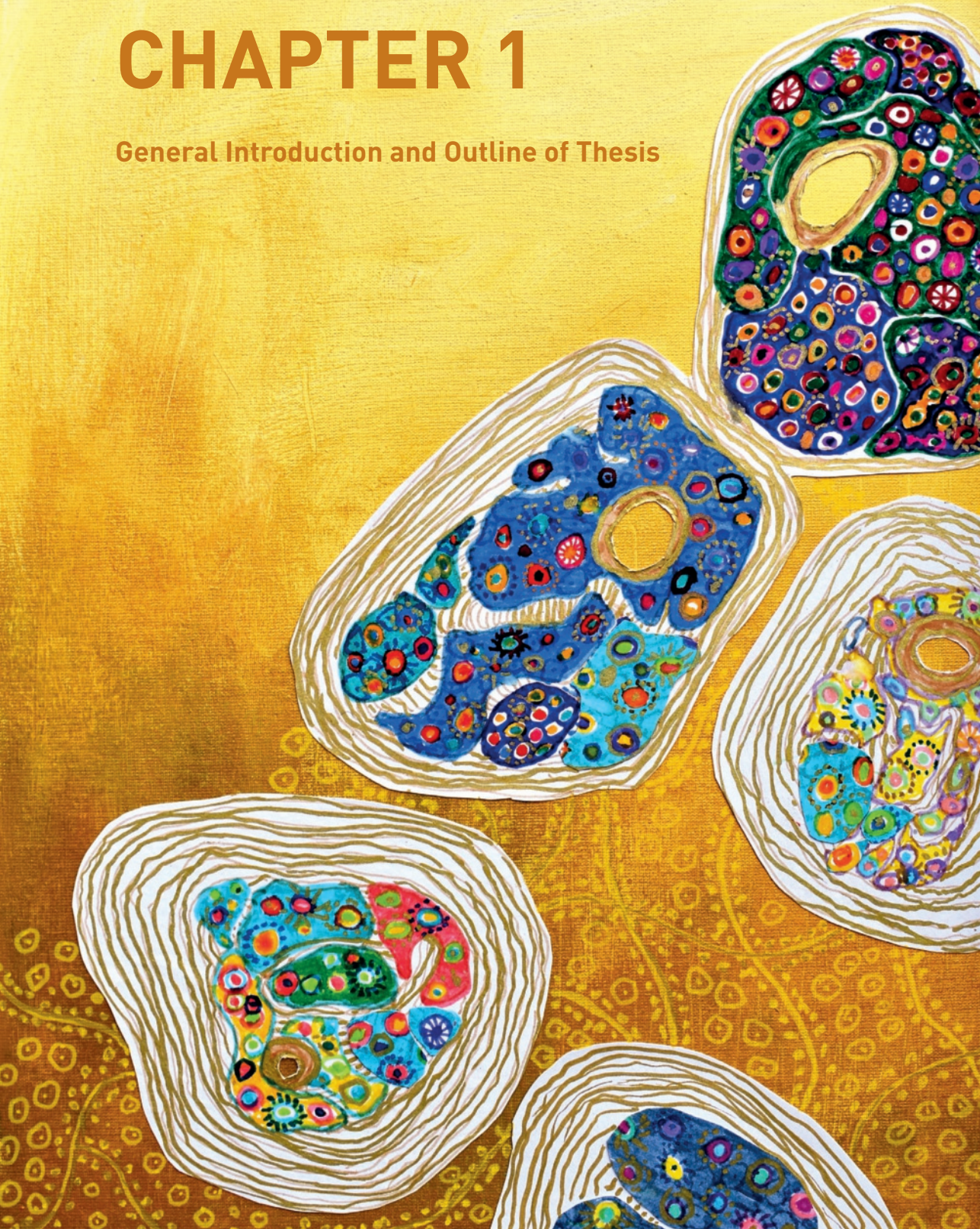
TABLE OF CONTENTS

CHAPTER 1

General Introduction and Outline of Thesis



SARCOPENIA

Almost a century ago, the age-related loss of muscle mass was first recognized by Macdonald Critchley (1), after which it took 58 years before an official term was given to this phenomenon. Prof. Irwin Rosenberg proposed that by using Greek terms, involuntary loss of skeletal mass might be taken more seriously and combined two Greek terms ''sarx'' (flesh) and ''penia'' (loss) to describe this observation (2). This strategy worked; since the introduction of the term ''sarcopenia'' emerging evidence on the extent of the problem and its negative health consequences was published in scientific journals.

Already after the 30th year of life, muscle mass starts to marginally decrease with approximately 0.4% per year (3). The decline of muscle mass accelerates with higher age. In people aged 75 year or older, muscle mass is lost at a rate of 0.6-1.0% per year (4). The loss of muscle mass is accompanied by a much more rapid loss of muscle strength (5). The decline in muscle strength after the age of 75 year is 2.5-4.0% per year (4). As a result of increasing life expectancy across the globe, the subpopulation of older adults is growing fast (**Figure 1**). In Europe 25% of the population is already aged ≥60 years and that proportion is estimated to reach 35% in 2050 (6). Luckily, these numbers also include vital, physically active older adults. To maintain their vital status and the ability to live at home, more research is needed to assess if we can maintain or even improve muscle mass and function in this specific sub-population of community-dwelling older adults.

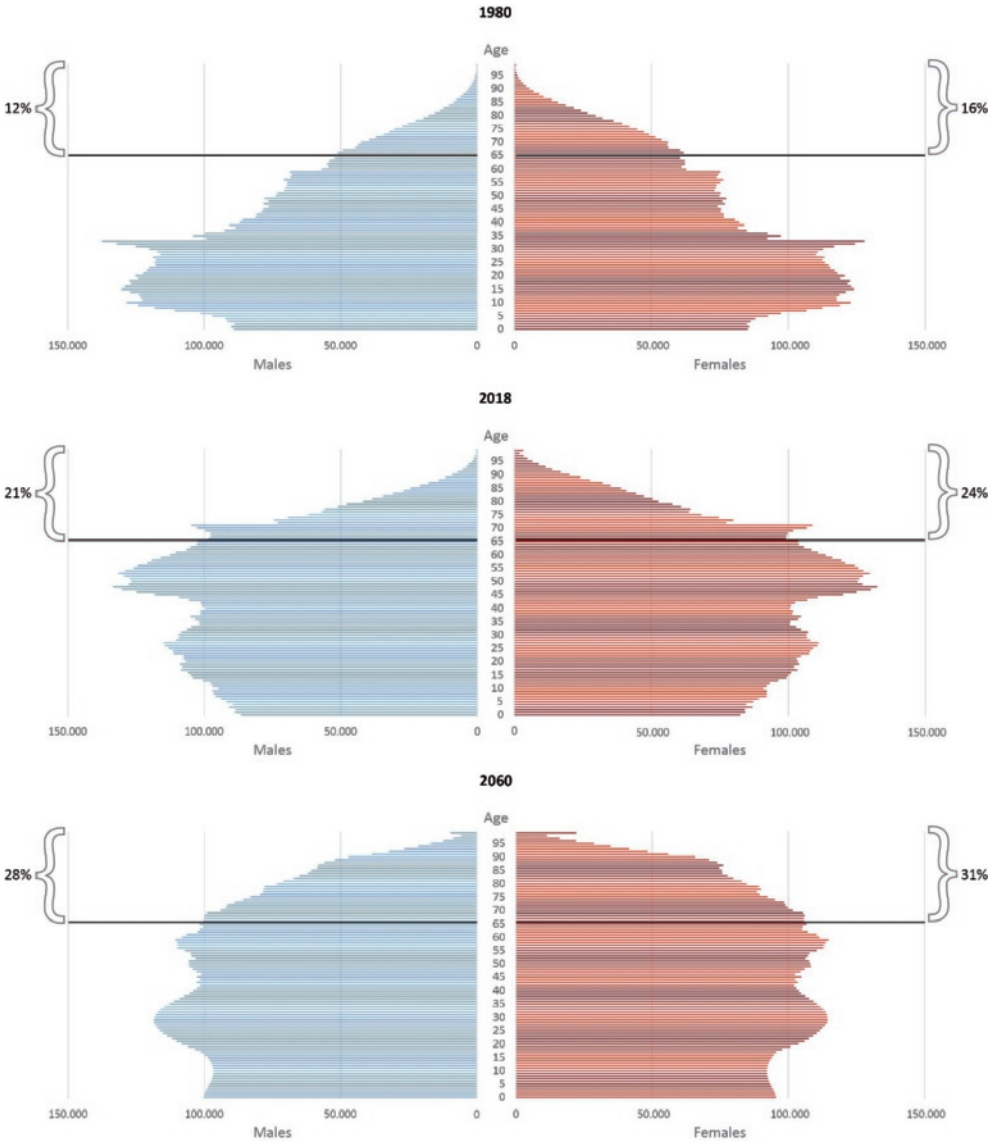


Figure 1. Dutch population pyramid of approximately 40 years ago: 1980, the present: 2018 and the expected population in 40 years: 2060, in which the percentage of people aged 65 years or older is increasing rapidly (Source: Centraal Bureau voor Statistiek (CBS) (7, 8)).

Mechanisms behind age-induced loss of muscle mass

Muscle proteins are constantly synthetized and broken down at a rate of 1-2% per day to maintain good quality of skeletal muscle tissue. The quantity of muscle mass is maintained when muscle protein synthesis and muscle protein breakdown are in balance. Rates of muscle protein synthesis are regulated predominantly by responsiveness to anabolic stimuli, such as physical activity or food intake (9, 10). The muscle protein synthetic response to anabolic stimuli is proposedly attenuated in the older adults as shown in **Figure 2** (11).

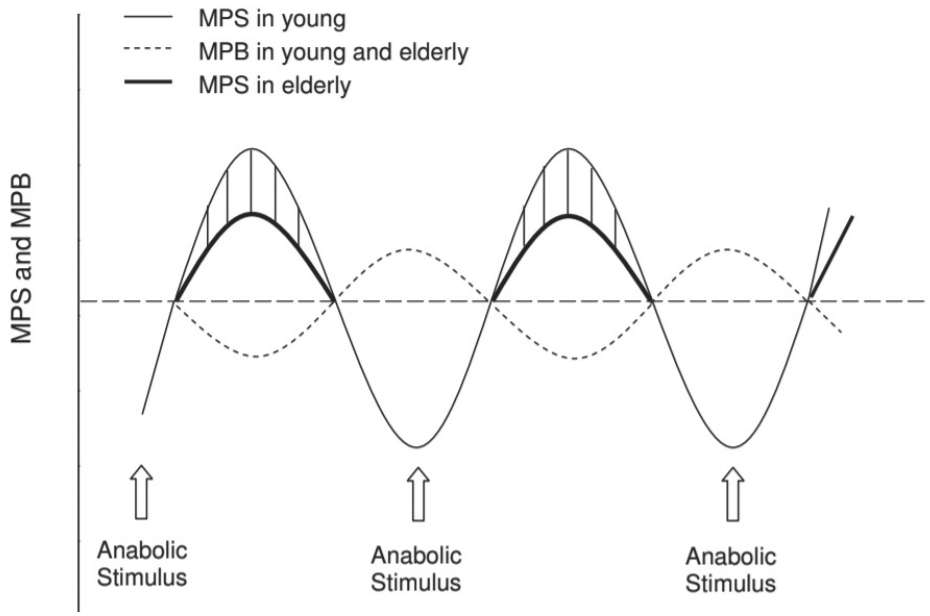


Figure 2. Schematic representation of muscle protein synthesis (MPS) and muscle protein breakdown (MPB) in young *versus* older adults in response to anabolic stimuli (exercise and/or amino acid ingestion) (Source: Breen & Philips (11)).

Consequently, during prolonged periods of decreased muscle protein synthesis, loss of muscle mass occurs (12, 13). Several factors may explain this anabolic resistance to stimuli (14). First, possibly impairments in protein digestion and amino acid absorption and elevated splanchnic extraction (i.e. the retention of dietary amino acids by the gut and liver for their own needs) results in a reduced amount of amino acids entering the circulation (14, 15). Furthermore, less amino acids might be delivered to and taken up by the muscle, because of an attenuated regulation of amino acid transporters, a decreased insulin-mediated capillary recruitment and limitations in the postprandial muscle perfusion (14, 16). Another explanation may be

the lower amount of the anabolic signaling protein, mammalian target of rapamycin complex I (mTORC1), found in older individuals, which can lead to a reduced capacity to respond to anabolic stimuli (17). Finally, the systemic chronic low-grade inflammation in older adults recently received more attention. The low-grade inflammation might not only attenuate the muscle protein synthesis in response to physiologic stimuli, but also increase muscle protein breakdown (18). Evidently, several processes at different levels seem to contribute to age-related loss of muscle mass.

Consequences of loss of muscle mass

The loss of muscle mass and the concomitant reduced muscle strength is accompanied by a decline in physical performance in older adults (19). With large involuntary loss of muscle mass and strength also the risk of falls increases (20). Moreover, skeletal muscle is responsible for ~30% of the resting energy expenditure and accounts for ~70-80% of glucose disposal after a meal. Therefore, with a reduced muscle mass total energy expenditure decreases which may lead to an enhanced fat accumulation if dietary patterns remain unchanged (21). So a secondary effect of sarcopenia is the increased risk for obesity (22), hyperlipidemia and hypertension (21), cardiovascular diseases (21), insulin resistance and thus higher prevalence of type 2 diabetes (21). Furthermore, muscle contractions give large voluntary loads on bone mass and are therefore essential for bone modeling and remodeling. Hence, reduced muscle mass or strength could also induce osteoporosis (22). All consequences of sarcopenia could lead to (longer) hospitalization (23) or institutionalization and consequently higher healthcare costs (24). Moreover, with the loss of muscle mass, loss of independence and an overall reduction of quality of life is at stake in the aging population (24). It is therefore very important to prevent or delay the age-related loss of muscle mass.

Factors that may counteract the loss of muscle mass

Several factors have been proposed that may affect the loss of muscle mass. The factors that are assessed in this thesis are described below (**Figure 3**).

Physical activity

Physical activity is a vital component to maintain muscle mass with advancing age (13). A physically active lifestyle is associated with a larger muscle mass and function (13). The adherence to an active lifestyle seems to maintain the sensitivity of older skeletal muscle to dietary amino acids and might suppress the catabolic inflammatory cytokines in the muscle (13, 25). Habitual physical activity does not need to be particularly intense to attenuate age-related muscle loss (13). Moreover, exercise bouts can enhance muscle protein synthesis rates with peaks in the first 3 hours after exercise, and the increased muscle protein synthesis rates may persist 18 to 24 hours after an exercise bout (26). Resistance exercise bouts have

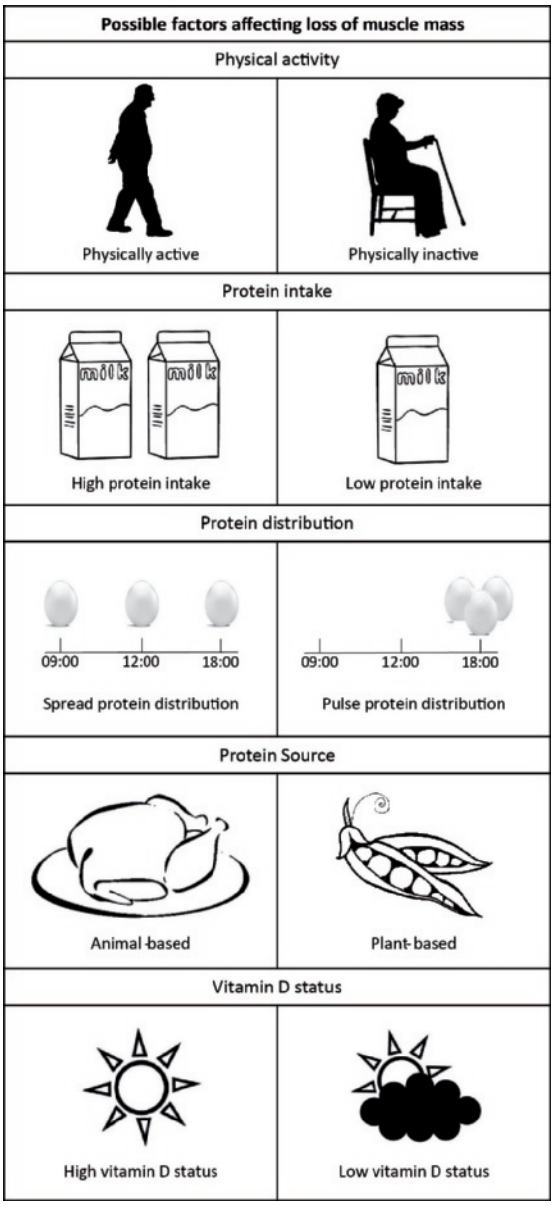


Figure 3. Possible factors affecting loss of muscle mass

shown to be the most potent stimulus to increase muscle mass and strength (13). Lately, the beneficial effect of endurance exercise on sarcopenia prevention has received more attention. Prolonged endurance exercise seems to attenuate age-related reductions in muscle strength as well (27). Moreover, even moderate-intensity exercise such as walking can improve the amino-acid delivery to the muscles in older adults (28). Although, endurance exercise bouts

seem to be less effective than resistance exercise bouts (29), the combination of resistance and endurance exercise training has been proposed as the most effective strategy to counteract the loss of muscle mass, because both myofibrillar and mitochondrial protein synthesis are been stimulated by the combined types of exercise (30).

Protein

Protein amount. Adequate protein intake is essential for the maintenance of muscle mass. General guidelines prescribe a protein intake of 0.8 grams per kilogram bodyweight per day (g/kg/d) for adults (31). Given the relative anabolic resistance for muscle protein synthesis to dietary protein, older adults require a higher protein intake compared to younger individuals to counteract the loss of muscle mass and function (32-34). The PROT-AGE study group proposes to increase the protein intake for adults above 65 years of age to 1.0-1.2 g/kg/d (26). Moreover, physically active older adults should consume ≥1.2 g/kg/d in order to comply with the synergistic effects of exercise and protein intake on muscle protein synthesis (26).

Protein source. The anabolic properties (*i.e.* quality) of specific protein sources are determined by their (essential) amino acid profile, digestibility and the amount of splanchnic extraction which determines the postprandial bioavailability (35). Indispensable amino acids cannot be synthesized *de novo* and are therefore essential to include in the diet. Dispensable amino acids can be synthesized in the body from other carbon sources and are therefore non-essential. Some amino acids cannot be derived *de novo* only in certain circumstances and are therefore conditionally indispensable (9). Animal-based proteins generally contain more essential amino acids, have smaller splanchnic extraction and are better digestible than plant-based proteins and therefore have a superior muscle protein synthetic response (36). However, with animal-based proteins automatically more saturated fats are co-ingested that can have negative health consequences on mainly the heart and blood vessels (37). Thus, a good ratio of animal- and plant based proteins is important for optimal health outcomes.

Protein distribution. Western diets are typically unbalanced in the distribution of protein over the day, which is expected to be especially detrimental in older adults. Older adults display an anabolic resistance to low amounts of dietary amino acids and the amounts consumed habitually at breakfast and lunch in the western diets are often much lower than at dinner (13). Incorporating doses of 25-30 g protein at the main meals in the diet of older adults has been suggested as a promising strategy to counteract the attenuated post-prandial muscle protein synthesis (38, 39). This spread-feeding pattern might ensure an optimal continuous stimulus of protein synthesis over the day, since the increased muscle protein synthesis response after protein intake lasts for around 4-5 hours after ingestion (36). In contrast, other studies found promising results of a pulse-feeding pattern in which a high-protein meal (i.e. ~ 70-80% of the total protein intake consumed at midday) might saturate splanchnic sequestration leading

to a higher availability of amino acids for muscle protein synthesis (40, 41). Thus, the optimal strategy still has to be determined.

Vitamin D

The effect of vitamin D status on muscle mass and function has received increasing attention in the past years. Vitamin D status, which is commonly assessed as serum 25-hydroxyvitamin D (25(OH)D) concentration, is positively correlated with muscle mass and function (42, 43). Moreover, prospective, observational and interventional studies have shown that higher serum 25(OH)D concentrations are associated with a decreased loss of muscle mass, muscle strength and function (44-47). The exact mechanisms behind these positive effects are not clear yet, but several pathways have been proposed. First, the binding of the active form of vitamin D, $1,25(OH)_2D$, to the vitamin D receptor (VDR) enhances circulating insulin-like growth factor-1 (IGF-1) that might induce muscle cell proliferation and growth (48). Moreover, not only IGF-1 is enhanced by the binding, but a range of proteins, including those involved in calcium metabolism. Calcium is a critical modulator of muscle function (48). Finally, studies in rats have shown that a higher vitamin D status inhibits the rate of muscle protein breakdown in the skeletal muscle (49).

Current questions

Most studies about the effects of habitual protein intake and protein supplementation on muscle mass and function are currently being performed in (pre-)frail elderly, whereas evidence about the optimal protein intake strategy is lacking for non-frail, physically active elderly. Therefore, we are specifically interested in the effects of habitual protein intake and protein supplementation on muscle characteristics in non-frail, physically active, community-dwelling older adults. Such information may give more insight in the potential of protein interventions to prevent or delay loss of muscle mass and function in physically active older adults and consequently extend their ability to live healthy and independently at home.

Outline of the thesis

In **chapter 2** we assessed the habitual protein intake of physically active older adults and the prevalence of the population that met the protein intake guidelines. This is important as sufficient protein intake is needed to utilize the benefits of the exercise-induced enhanced muscle protein synthesis and, thus, to prevent age-related muscle mass loss.

The beneficial effect of enhancing protein intake with supplementation has shown conflicting results, which may partly be explained by the different target groups. Whereas in frail older adults often promising results are found, in non-frail community-dwelling older adults more conflicting results are present. In **chapter 3** we performed a meta-analysis on exclusively non-frail community-dwelling older adults to assess the effect of protein supplementation

on lean body mass, muscle strength and physical performance. Moreover, we assessed the superior effects of protein supplementation during resistance exercise training on muscle characteristics in exclusively non-frail community-dwelling older adults.

Habitual protein intake, protein distribution and physical activity levels of participants can also affect muscle characteristics. In **chapter 4** we investigated whether protein intake and protein intake distribution are associated with muscle strength, physical function and quality of life in community-dwelling older adults while additionally accounting for the role of physical activity.

In **chapter 5** we assessed the effects of 12 weeks of daily protein supplementation on lean body mass, muscle strength and physical performance in physically active older adults with a low habitual protein intake using a randomized double-blinded controlled trial.

In **chapter 6** we assessed another contributing factor for optimal muscle health, vitamin D. We assessed the vitamin D status in different age subgroups of physically active older adults. Moreover, we assessed which determinants contributed to vitamin D status.

Finally, in **chapter 7** we discuss the concepts of each chapter and how they related to each other, we describe how they support the current body of literature and discuss the most important findings and implications and provide future directions for research in this area.

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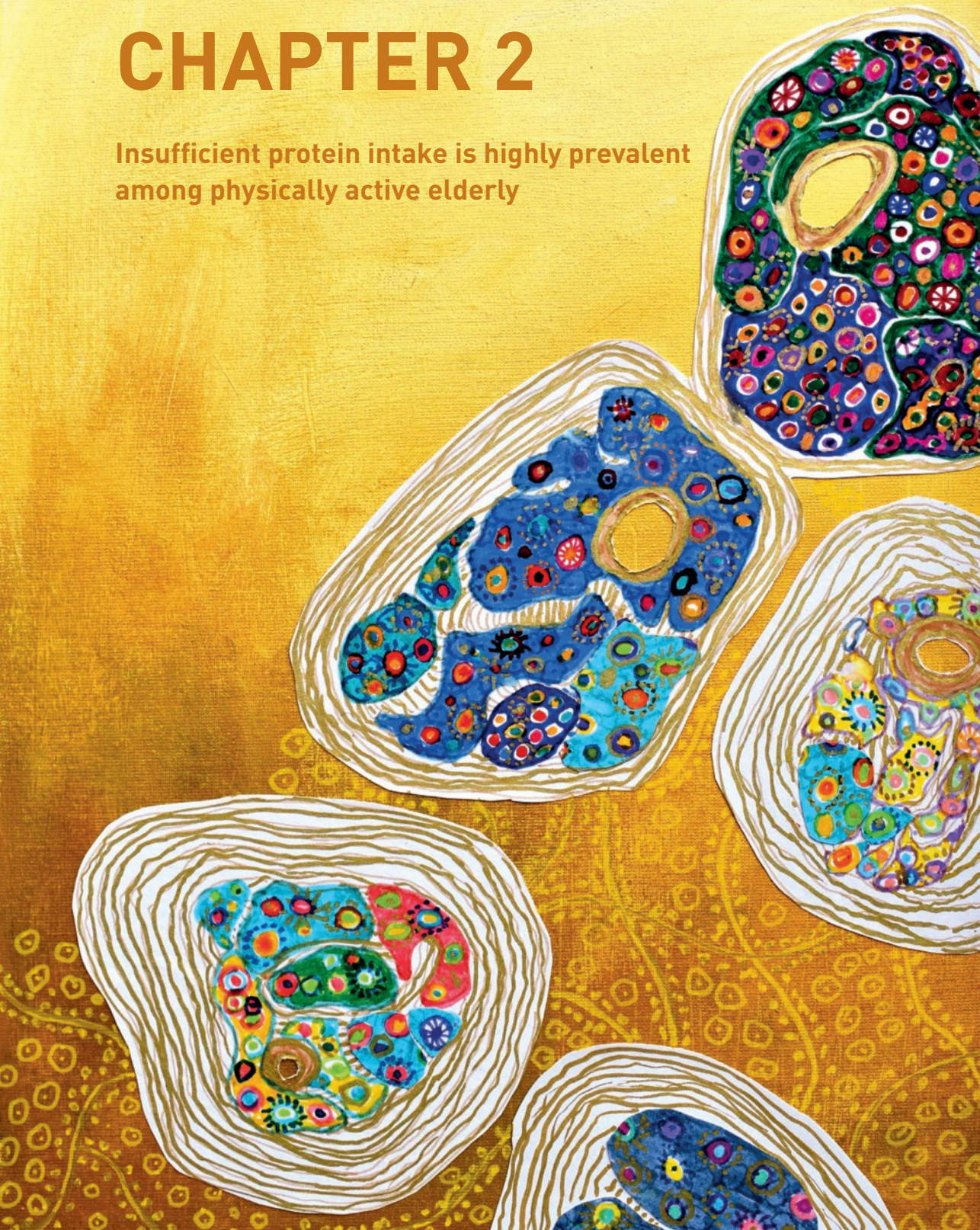
CHAPTER 2

Insufficient protein intake is highly prevalent among physically active elderly



ABSTRACT

Objectives: Sufficient protein intake and habitual physical activity are key factors in the prevention and treatment of sarcopenia. In the present study, we assessed habitual dietary protein intake and the contribution of animal proteins in male *versus* female physically active elderly and identified determinants of protein intake.

Design: a cross-sectional study.

Setting: the study was performed within the *Nijmegen Exercise Study*.

Participants: physically active elderly ≥ 65 yrs.

Measurements: Physical activity was assessed using the SQUASH questionnaire and expressed in Metabolic Equivalent of Task hours per week (METhr/wk). Dietary protein intake was determined using a validated food frequency questionnaire (FFQ). Multivariate linear regression analysis was used to determine whether age, sex, educational level, smoking, alcohol intake and physical activity were associated with protein intake (g/kg/d).

Results: A total of 910 participants (70±4 yrs, 70% male) were included and reported a habitual physical activity level of 85.0±53.5 METhr/wk. Protein intake was 1.1±0.3 g/kg/d with 57% animal-based proteins for males, and 1.2±0.3 g/kg/d with 59% animal-based proteins for females (both $P<0.05$). In total, 16%, 42% and 67% of the male elderly and 10%, 34% and 56% of the female elderly did not meet the recommended protein intake of 0.8, 1.0 and 1.2 g/kg/d, respectively. Female sex ($\beta=0.055$, $P=0.036$) and more physical activity ($\beta=0.001$, $P=0.001$) were associated with a higher daily protein intake (g/kg/d).

Conclusion: The majority of physically active elderly and in particular males (i.e. 67%) does not reach a protein intake of 1.2 g/kg/d, which may offset the health benefits of an active lifestyle on muscle synthesis and prevention of sarcopenia. Intervention studies are warranted to assess whether protein supplementation may enhance muscle mass and strength in physically active elderly.

INTRODUCTION

Physical activity and sufficient protein intake are key factors in the prevention and treatment of sarcopenia (1, 2). Evidence shows that elderly require a higher protein intake compared to younger individuals to maintain muscle mass and function, and to counteract sarcopenia (2-4). Although general guidelines prescribe a protein intake of 0.8 grams of protein per kilogram body weight per day (g/kg/d) (5), it has been suggested that physically active elderly need a protein intake ≥1.2 g/kg/d to prevent age-related loss of muscle mass and function (6-8). To our knowledge, information on habitual dietary protein intake of physically active elderly and possible differences between males and females is currently lacking. Therefore, the objective of this study is to determine habitual dietary protein intake and the contribution of animal proteins in male *versus* female physically active elderly. Moreover, we investigated which demographic and lifestyle factors contribute to habitual protein intake.

METHODS

Study population

Participants aged ≥ 65 years were recruited via the *Nijmegen Exercise Study* (Study-ID: NL36743.091.11) (9) and were invited to complete a questionnaire about subject characteristics, physical activity (10) and dietary intake (11, 12). All subjects gave written informed consent prior participation. The study was approved by the Medical Ethical Committee of the Radboud University Medical Center.

Descriptive characteristics and covariates

Data on demographic characteristics (age, sex, level of education), anthropometric measures (height and weight) and lifestyle factors (smoking, alcohol, and physical activity) were collected. Physical activity was assessed using the validated Short Questionnaire to Assess Health Enhancing Physical Activity (SQUASH) (10). Physical activity volumes were expressed as metabolic equivalent of task hours per week (METhr/wk), and calculated for sport, commuting and leisure time activities. Based on international physical activity recommendations (500 - 1000 METmin/wk) (13), participants with a physical activity <8.3 METhr/wk were excluded from further analysis.

Dietary intake

A validated online Food Frequency Questionnaire (FFQ) contained 180 food items to estimate habitual dietary intake over the past month, including protein intake (11, 12). Intake of total energy and nutrients was calculated using the Dutch Food Composition Database of 2010

(NEVO). Dietary misreporting was evaluated using Goldberg cut-off values (14, 15) and under- or over-reporting subjects were excluded from further analyses.

Statistical analysis

Participant characteristics were displayed as mean ± SD for continuous variables and as counts with percentages for categorical variables. P-values for differences between males and females were derived with an independent sample t-test or a Chi-square test. Associations between possible determinants (i.e. age, sex, educational level, smoking, alcohol intake and physical activity) and total daily protein intake were analyzed in a multivariate linear regression (forced entry method). Analysis was conducted using SPSS 22.0 and a two-sided level of significance was set at $p < 0.05$.

RESULTS

Complete data were available for 1005 elderly. Sixty-eight (7%) participants were underreporting, whereas 14 participants were overreporting (1%) and therefore excluded from further analysis. Another 13 (1%) participants were excluded based on the fact that they did not meet the criteria for a physically active lifestyle (<8.3 METhr/wk). Descriptive characteristics of the remaining 910 physically active participants are summarized in **Table 1**. Significant differences between males and females were present for age, weight, BMI, alcohol intake and educational level.

Table 1. Descriptive characteristics of our cohort of physically active males and females.

	Total group (*n* = 910)	**Male** (*n* = 636, 70%)	**Female** (*n* = 274, 30%)	***P*-value**
Age (yr)	70 ± 4	70 ± 4	69 ± 4	<0.001
Weight (kg)	76 ± 12	80 ± 10	65 ± 9	<0.001
BMI (kg/m^2)	24.8 ± 2.9	25.3 ± 2.7	23.7 ± 3.0	<0.001
Physical activity (METhr/wk)	85.0 ± 53.5	86.7 ± 53.9	81.2 ± 52.5	0.16
Currently smoking, n (%)	47 (6)	35 (6)	12 (5)	0.59*
Alcohol intake (g/d)	15.4 ± 15.2	17.7 ± 16.1	10.2 ± 11.2	<0.001
Educational level				<0.001*
Low, n (%)	353 (40)	217 (35)	136 (52)	
Intermediate, n (%)	214 (24)	157 (25)	57 (22)	
High / academic, n (%)	316 (36)	245 (40)	71 (27)	

Data are presented as mean ± SD or number (percentage) of participants. *Derived from a Chi-square test. BMI; body mass index, MET; metabolic equivalents of task.

Average daily energy intake was 2350 ± 580 kcal for males and 1900 ± 447 kcal for females (*P* = <0.001), of which 15 ± 2% and 16 ± 2% (*P* = <0.001), respectively, was derived from protein sources. Animal-based proteins contributed for 57 ± 10% to total protein intake in males and for 59 ± 10% in females (*P* = 0.016). Protein intake was on average 1.1 ± 0.3 g/kg/d for males, and 1.2 ± 0.3 g/kg/d for females (*P* = 0.014). A total of 16%, 42% and 67% of male elderly were below the recommended protein volumes of 0.8, 1.0 and 1.2 g/kg/d, respectively, whereas 10%, 34% and 56% of female elderly were below 0.8, 1.0 and 1.2 g/kg/d, respectively (*P* = 0.010 **Figure 1**).

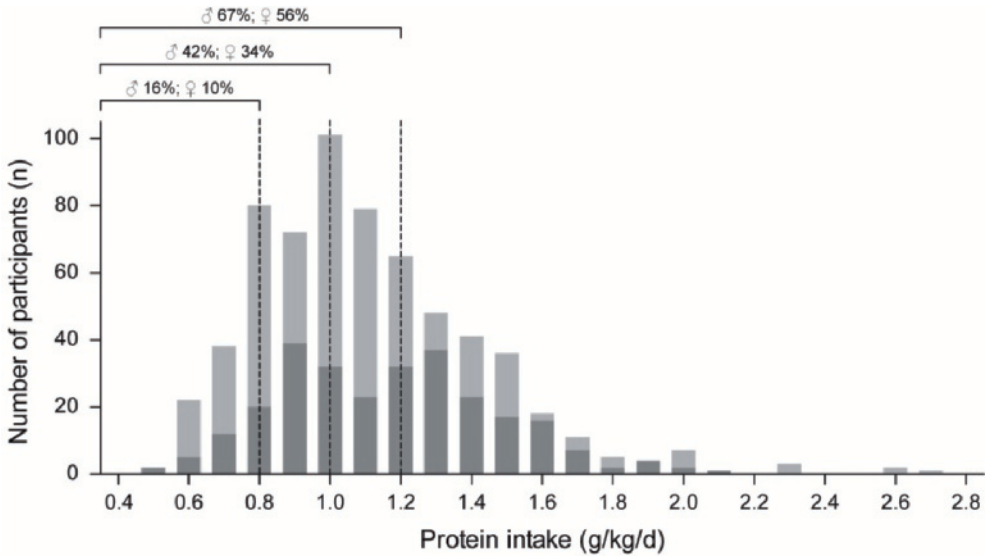


Figure 1. Frequency distribution of protein intake (g/kg/d) for males (light bars, ♂) and females (dark bars, ♀). Mean protein intake was 1.1 ± 0.3 g/kg/d for males and 1.2 ± 0.3 g/kg/d for females (*P* = 0.010). A total of 16%, 42% and 67% of male elderly were below a protein intake threshold of 0.8, 1.0 and 1.2 g/kg/d, respectively, whereas 10%, 34% and 56% of female elderly were below a protein intake threshold of 0.8, 1.0 and 1.2 g/kg/d, respectively (*P* = 0.010). These findings suggest that the majority of physically active elderly, and in particular males, have an insufficient protein intake, which may offset the benefits of a physically active lifestyle on muscle synthesis.

Female sex (β = 0.055, *P* = 0.036) and more physical activity (β = 0.001, *P* = 0.001) were significantly associated with total daily protein intake (g/kg/d) in the multivariate regression model (R^2=0.027), whereas age, smoking, education level and alcohol intake were not associated with protein intake.

DISCUSSION

To the best of our knowledge, we performed the first study to assess habitual protein intake in very physically active elderly. The average protein intake was 1.1 ± 0.3 g/kg/d in males, and 1.2 ± 0.3 g/kg/d in females which is similar to earlier reported protein intakes in community-dwelling elderly (16). Although such a protein intake exceeds the recommendations for the general population (>0.8 g/kg/d), this may not be enough for physically active elderly. Physical activity stimulates prolonged utilization of circulating amino acids for muscle protein synthesis (17, 18) but also results, to a smaller extent, in protein degradation. A protein intake of ≥1.2 g/kg/d is recommended for physically active elderly (7) in order to obtain a positive protein balance (19). The majority of our physically active cohort (67% in males and 56% in females) failed to meet this recommendation and consequently may have had a negative protein balance. Targeted protein interventions may assist physically active elderly to exceed a habitual protein intake ≥1.2 g/kg/d and subsequently postpone or counteract the age-related loss of muscle mass and function.

More detailed information about the protein source of physically active elderly provides vital information on which aspects of the protein intake could be improved. Male and female elderly had an animal-based protein intake of 57% and 59% respectively. The intake of animal-based proteins results in a superior muscle protein synthetic response compared to plant-based proteins, as a result of a better digestibility, smaller splanchnic extraction and better essential amino acid composition in animal-based proteins (20). However, an abundant intake of animal-based proteins with co-ingestion of more saturated fats could have negative health effects (e.g. cardiovascular disease and decreased bone health) (21). Ingestion of multiple plant-based protein sources could provide a more balanced amino acid profile (20). Therefore, protein strategies focused on an optimized balance of animal/plant ratio with diverse plant-based protein sources could be of particular interest for muscle protein synthesis in physically active elderly.

Finally, we found that female sex and higher physical activity levels were positively associated with total daily protein intake. Since males in general have a higher muscle mass compared to females (22), physically active males ≥ 65 years might benefit even more from strategies to improve the protein intake.

A limitation of the present study may be the use of the FFQ to assess habitual protein intake, as previous studies have suggested that 24 hour recalls or dietary recalls have a higher precision in estimating the distribution of protein intake (23). However, the FFQ used in the present study was very elaborate (180 items) and primarily focused on protein intake. Moreover, compared to the urinary biomarker nitrogen (N), which is often used as the estimate of true intake of

protein, a smaller variation is found and thus a smaller distribution with the FFQ (24). Hence, our findings may even represent an underestimation of the true prevalence of individuals not meeting the habitual protein intake recommendations.

In conclusion, the majority of physically active elderly, and in particular males, have a protein intake below 1.2 g/kg/d, which may offset the benefits of an active lifestyle on muscle synthesis. Future research in physically active elderly is needed to assess if age-related loss of muscle mass can be postponed or counteracted with optimized protein intake interventions.

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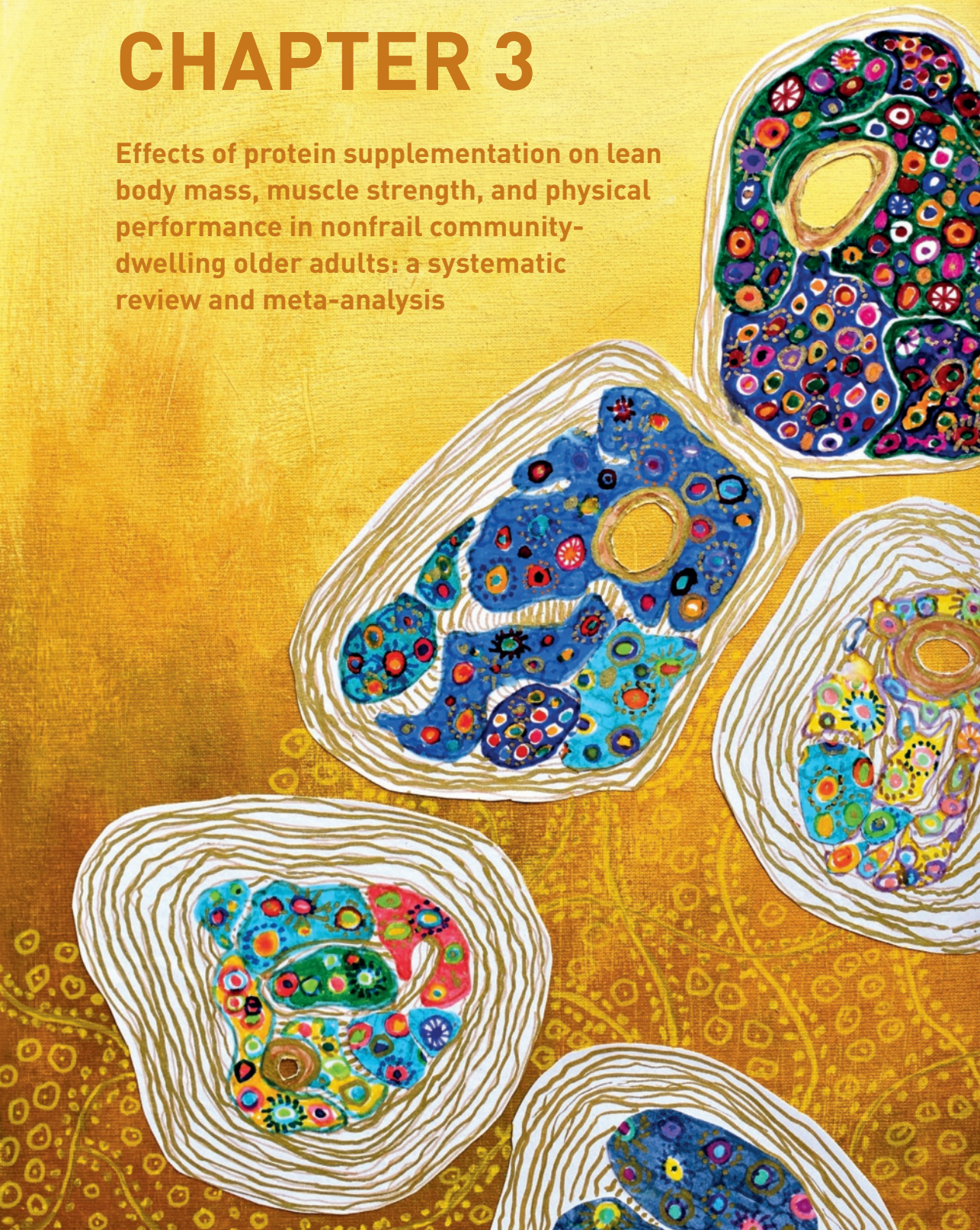
CHAPTER 3

Effects of protein supplementation on lean body mass, muscle strength, and physical performance in nonfrail community-dwelling older adults: a systematic review and meta-analysis



ABSTRACT

Background: Increasing protein intake has been suggested as an effective strategy to ameliorate age-related loss of muscle mass and strength. Current reviews assessing the effect of protein supplementation are strongly influenced by the inclusion of studies with frail older adults.

Objectives: We assessed the effect of protein supplementation on lean body mass, muscle strength, and physical performance in exclusively nonfrail community-dwelling older adults. Moreover, we assessed the superior effects of protein supplementation during concomitant resistance exercise training on muscle characteristics.

Design: A systematic literature search was conducted on Pubmed, Embase and Web of Science up to May 15th 2018. We included randomized controlled trials that assessed the effect of protein supplementation on lean body mass, muscle thigh cross-sectional area, muscle strength, gait speed and chair-rise ability and performed random-effects meta-analyses.

Results: Data from 36 studies with 1682 participants showed no significant effects of protein supplementation on changes in lean body mass (standardized mean difference (SMD): 0.11; 95% CI -0.06, 0.28), handgrip strength (SMD: 0.58; 95% CI: -0.08, 1.24), lower extremity muscle strength (SMD: 0.03; 95% CI -0.20, 0.27), gait speed (SMD: 0.41; 95% CI -0.04, 0.85) or chair-rise ability (SMD: 0.10; 95% CI -0.08, 0.28) compared with a control condition in nonfrail community-dwelling older adults. Moreover, no superior effects of protein supplementation were found during concomitant resistance exercise training on muscle characteristics.

Conclusions: Protein supplementation in nonfrail community-dwelling older adults does not lead to increases in lean body mass, muscle cross-sectional area, muscle strength, or physical performance compared with control conditions, nor does it exert superior effects when added to resistance exercise training. Habitual protein intakes of most study participants were already sufficient and protein interventions differed in terms of type of protein, amount and timing. Future research should clarify what specific protein supplementation protocol is beneficial for nonfrail community-dwelling older adults with low habitual protein intake.

INTRODUCTION

Age-related, progressive loss of muscle mass and strength, referred to as sarcopenia, is one of the major determinants of disability in older adults (1). The depletion of muscle mass predisposes older adults for bone fractures (2) and the development of chronic metabolic diseases, such as type 2 diabetes (2, 3) and obesity (2), leading to substantial increases in health care costs (4, 5).

To prevent or delay the age-associated decline of physical capabilities, several strategies have been proposed to counteract the loss of muscle mass and muscle strength. Resistance exercise training is a well-established method to elicit an anabolic response and consequent gains in muscle mass and strength in older adults from the general population (6-8). Moreover, a high dietary protein intake was associated with an attenuated loss of muscle mass in community-dwelling older adults (9, 10).

Several systematic reviews and meta-analyses assessed the effects of protein supplementation on lean body mass, muscle strength, and/or physical performance (11-17), but these reviews were strongly influenced by the inclusion of studies with frail older adults. For example, randomized controlled trials (RCTs) including frail older adults found beneficial effects of protein supplementation on muscle characteristics (18, 19), whereas RCTs performed in healthy older adults did not find such a beneficial effect (20, 21). A systematic review on the effect of protein supplementation on muscle characteristics and physical performance in community-dwelling older adults is lacking but of high relevance considering the growing population of vital older adults.

The aim of this systematic review and meta-analysis was to assess the effect of protein supplementation on lean body mass, muscle strength and/or physical performance, in nonfrail community-dwelling older adults. Moreover, we aimed to assess the superior effect of protein supplementation during concomitant resistance exercise training. We hypothesized that protein supplementation alone does not have a beneficial effect on lean body mass, muscle strength, and physical performance, but that this may be overcome when combining resistance exercise training with additional protein supplementation.

METHODS

Search strategy and study identification

A systematic review was performed with the use of the Preferred Reporting Items for Systematic reviews and Meta-Analysis statement 2015 (PRISMA) (22). The Pubmed, Embase and Web of

Science databases were systematically searched for articles up to 15 May 2018. The following search strategy was used, with adaptation for each database: (Aged OR Middle aged OR Elderly OR Old* people OR Old* person* OR Old* adult* OR Old* population* OR healthy older adults) *AND* (Protein intake OR Protein supplement* OR Nutritional protein* OR Dietary Protein* OR Essential Amino Acids OR Milk Protein* OR Casein* OR Whey* OR Amino Acid*) *AND* (Muscle strength AND Skeletal muscle AND Handgrip strength OR Hand strength OR Grip strength OR leg press strength OR quadriceps strength OR Muscle mass OR fat free mass OR lean body mass OR lean tissue mass OR Muscle function OR Muscle quality OR physical condition OR physical function OR physical functionality OR physical activity OR physical active OR physical performance OR Physical working capacity OR physical capacity OR functional performance OR Mobility condition* OR Mobility active OR Mobility activity OR Mobility performance OR Muscles OR Muscle OR myofibril* OR Muscle protein synthesis*). To ensure we found all the articles for our secondary aim, we performed an additional search in which we extended our search with the Medical Subject Headings term *''AND* Resistance training''. Within this search RCTs were identified with validated methods for the different databases (23). For Pubmed the sensitivity- and precision-maximizing version was found most suitable, whereas for Embase the Cochrane Lefebvre was used, which was also adapted and used for Web of Science (23). Reference lists of included articles were manually checked for possibly eligible studies that were missed during the literature search (**Figure 1**).

Study selection

After elimination of duplicates, 2 reviewers (DSMtH and MAHN) independently screened study titles for eligibility with the use of the inclusion and exclusion criteria in the review protocol (**Table 1**). Subsequently, the abstracts of the remaining studies were screened and 96 studies were assessed in full text to determine whether the data could be added to the meta-analysis (Figure 1). Inter-reviewer disagreements were resolved through consensus or by consulting a third reviewer (MTEH). RCTs were deemed eligible if they conformed to the predetermined inclusion and exclusion criteria (Table 1). ''Older adults'' was defined as an average age of ≥50 y. Moreover, participants had to be nonfrail and community-dwelling. Studies (or study arms) with patient populations suffering from the following diseases- cancer, muscle diseases, lung diseases, kidney diseases, gastrointestinal diseases, diabetes, cardiovascular diseases, or immunodeficiency diseases- were excluded because of interference with muscle characteristics (24, 25). Furthermore, studies with participants that were hospitalized or immobilized or had an assisted-living situation were also excluded. Suitable interventions were those studies that used oral (multi-nutrient) protein supplementation or a mixture of ≥8 essential amino acid or protein-rich products. The intervention could be additional to the participants' normal diet or replace their normal diet. Studies in which the protein group and the control group both performed resistance exercise training were also included. Interventions with an energy intake restriction were excluded. The minimal duration of the intervention was set at 4 wk.

Studies that used ≥1 from the following outcome measures- lean body mass (or fat-free mass) measured with dual energy X-ray absorptiometry (DXA), hydrostatic weighing (deuterium oxide dilution), whole-body air plethysmogrophy (Bod Pod) or hydro densitometry (underwater weighing), thigh muscle cross-sectional area measured with computerized tomography or MRI, isometric upper body and lower extremity muscle strength and physical performance tests that determined gait speed and chair-rise ability- were included. Finally, non-English articles, conference proceedings, and articles with abstracts only or study protocols only were excluded.

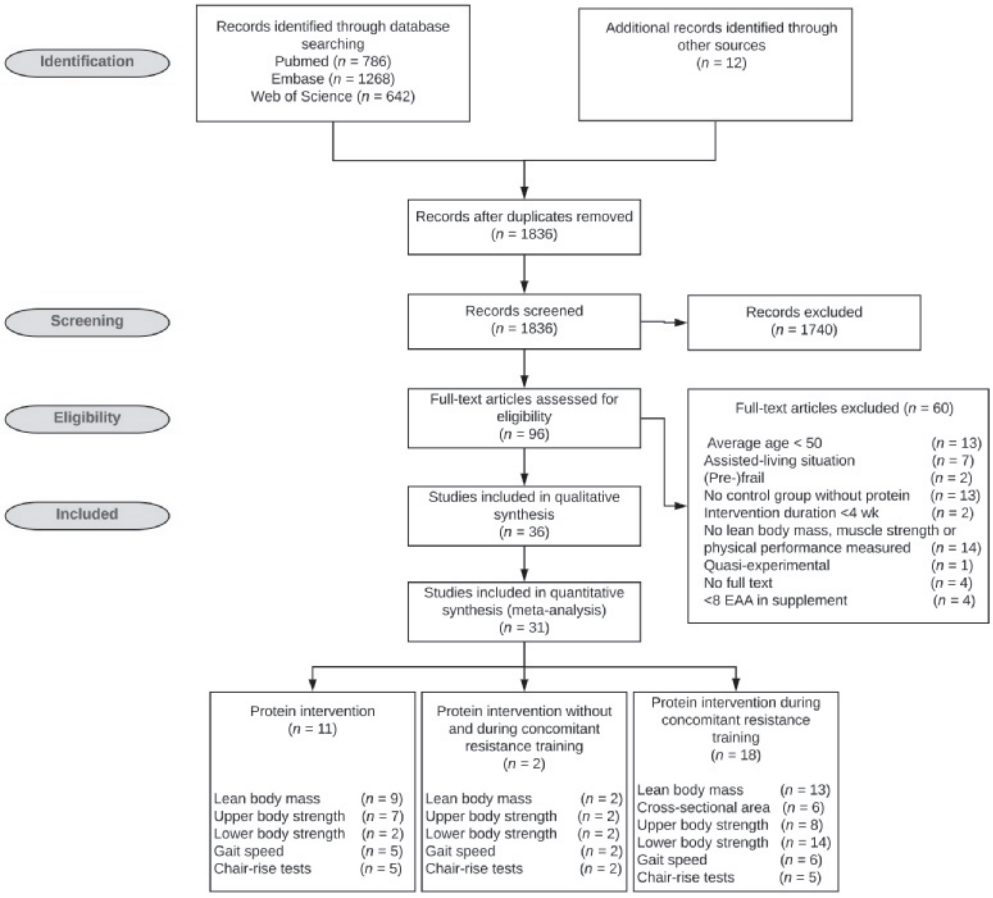


Figure 1. PRISMA flowchart of search strategy outcomes. EAA, essential amino acid; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analysis.

Table 1. Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Population	
- Mean age of > 50	- Hospitalized patients / immobilized individuals (bed rest, cast)
- Community dwelling	- Assisted-living situation
- Nonfrail older adults	- Participants with cancer, muscle diseases, lung diseases, kidney diseases, gastrointestinal diseases, diabetes, cardiovascular diseases or immunodeficiency diseases.
Intervention	
- All randomized controlled trials that used oral (multi-nutrient) protein or essential amino acid supplementation / nutritional products of any consistency	- Amino acid mix with <8 essential amino acids.
- Oral protein or amino acid supplementation can be additional to the participants' normal diet or replace their normal diet	- Dietary counseling only
- Effect of protein or amino acid supplementation is assessed and compared with a (different) control group.	- Intervention with energy intake restriction
- Studies in which the protein or amino acid group and the control group both performed resistance exercise training are also included. The supplementation should at least be consumed 3 times/wk (around resistance exercise training).	- Non-oral feeding
- Minimal duration of 4 wk	
Outcome	
- Lean body mass or fat-free mass	
- Thigh muscle cross-sectional area	
- Upper body or lower extremity muscle strength in kilograms	
- Physical performance (gait speed or chair-rise test)	
Other	
- English language	- Abstract only
- Full papers	- Conference proceedings
	- Study protocol / Letter to the editor

Data extraction

Data were extracted with the use of a predetermined data extraction file. Baseline and post-intervention data of lean body mass, muscle strength, and physical performance with corresponding SDs or SEs were independently recorded by 2 reviewers (DSMtH and MAHN). Thigh-muscle cross-sectional area was assessed. For muscle strength, upper body and lower extremity strength were assessed separately. When studies included multiple exercises to determine strength in the upper body or lower extremities, the exercise that measures the largest muscle group was used in the analysis (e.g. upper body: lat pull down > bench press > chest press > biceps curl > triceps extension > preacher curl, respectively and lower extremities: leg press > leg extension > leg curl, respectively). Physical performance tests were divided into walking tests that determined gait speed and therefore aerobic capacity and tests that targeted daily functionality, e.g. chair-rise tests. When studies included multiple walking or chair-rise tests, we used the following order for assessing the gait speed: 400-m walk > 6-min walk > 10-m walk > 6-m walk test >Short Physical Performance Battery gait speed > 4 square step test, respectively, and for chair-rise tests: TUG (Timed Up-and-Go) test > Short Physical Performance Battery chair rise test, 30-s sit-to-stand test > 5 times sit-to-stand test > 15-s step test, respectively. Other information gathered included publication year, sample size, participants' sex, age, BMI, and intervention details: duration, type of protein, amount, type of placebo, training frequency, type of training, and training intensity. When viable information (*i.e.* information about the results needed for inclusion in our meta-analysis) was missing, an attempt was made to request missing information from the authors by email (n = 14 studies; authors of n = 6 studies provided requested information). For 5 included studies results were depicted in figures, thus the data were extracted with the use of GetData Graph Digitizer software version 2.26. Two studies could still be included in some analyses, but not all of their outcomes could be included in the analyses because some viable information was missing.

Assessment of risk of bias in included studies

The quality of each eligible study was independently assessed by 2 reviewers (DSMtH and MAHN), with the use of the Downs and Black checklist (26) which is composed of 27 items for evaluating the risk of bias, based on the quality of reporting, external validity, internal validity (bias), internal validity (confounding/selection bias), and power. In total 32 points can be received with the original Downs and Black checklist with 27 questions. We excluded the final question (question 27) about power, because we performed a meta-analysis in which the independent power of the studies is irrelevant and underpowered studies will already be given less weight in the pooled analysis. On this excluded question 0-5 points could be received, therefore, our modified index could result in a score of 0 till 27. The quality scores were calculated and ranked on a 4-category scale: poor (<15), moderate (15–19), good (20–24) and excellent (≥25). Studies that were assessed as ''poor'' quality were excluded from the analysis. The Downs and Black checklist has been tested and found positive in terms of internal consistency, face

validity, content validity, criterion validity, and reproducibility. Only construct validity was not accomplished, something not achieved by most tools (27). The Downs and Black checklist has demonstrated good inter-rater reliability (r = 0.75) and test-retest reliability (r = 0.88) (26). Cohen's kappa values (K) for the inter-rater agreement between the 2 reviewers (28) were determined to be 0.85. This means a ''strong'' strength of agreement (28). Inter-reviewer disagreements were resolved by consensus.

Data synthesis and analysis

To account for potential heterogeneity between studies, a random-effects model (specified *a priori*) was used to determine the overall effect size of the intervention (protein supplementation) on lean body mass, muscle strength and physical performance. For each outcome measure of interest, a meta-analysis was performed to determine the pooled effect size in terms of the standardized mean difference (SMD) with corresponding 95% CI. A correlation of 0.5 between the outcomes measured in each study arm (i.e. protein compared with control) was assumed (29, 30). The magnitude of the SMD can be interpreted as small, SMD = 0.2; medium, SMD = 0.5; and large, SMD = 0.8 (31). When a study contained 2 protein intervention study arms (n = 5 studies) both study arms were included in the study analysis and individually compared against the control group. Analyses to assess the following comparisons- 1) protein supplementation with control condition and 2) protein supplementation during concomitant resistance exercise with control condition with resistance exercise- were performed. Forest plots were generated to illustrate the study-specific effect sizes along with a 95% CI and for each separate analysis the average duration of the intervention per participant was calculated in weeks. Cochran's Q statistic and the I^2 statistic were calculated to assess the degree of heterogeneity across studies. The Q statistic indicates statistically significant heterogeneity at $P < 0.10$. The I^2 statistic reflects the percentage of the observed between-study variability. An $I^2 > 50\%$ is considered to represent substantial heterogeneity (32). Publication bias was assessed via visual analysis of the funnel plot asymmetry with the use of the ''trim and fill'' and ''Classic fail 'n safe'' algorithms. To assess whether any individual studies included in the meta-analysis had a disproportionate effect on the results, sensitivity analysis was performed via the ''remove-one'' analysis. All calculations and plots were performed in CMA-2 (Comprehensive Meta-analysis version 2, 2011, Biostat, Englewood, NJ).

RESULTS

Selection of studies for the meta-analysis

The original search resulted in 2708 studies. Twelve additional studies were found from the reference lists of the included full-text papers. After removal of duplicates and elimination of papers based on the eligibility criteria, 36 studies were included of which 31 studies

presented unique data not shown in other studies included. A total of 11 studies assessed lean body mass, upper body strength, lower body strength, gait speed and/or chair-rise ability. Moreover, 18 studies assessed the additional effect of protein intake compared with controls on these variables while performing resistance exercise training. One study compared the effect of protein supplementation in 4 groups: protein compared with controls in groups without concomitant resistance exercise training and protein compared with controls in groups during concomitant resistance exercise training (33) and another study assessed the effect of protein supplementation first without resistance training and subsequently during concomitant resistance training (34) (Figure 1). Included studies were published from 1992 to 2018.

Quality assessment

Thirty-six studies were included in the quality assessment. The studies scored moderate to good on internal validity (bias) (mean ± SD: 5.8 ± 1.1, 83% of total); however, most of the studies scored low on external validity (1.7 ± 0.8, 57% of total). The quality of the majority of the studies was "moderate" or "good" (50% and 42%, respectively) and 3 studies were rated "excellent" (8%). The mean ± SD total score on the Downs and Black checklist was 19.6 ± 3.2 out of 27 (**Table 2**).

Cohort characteristics

Data from 1,682 participants were included in the analyses: 768 participants in studies that assessed protein supplementation compared with a control condition, 865 participants in studies that assessed the additional effect of protein intake compared with a control condition during concomitant resistance exercise training, and in 49 participants the effect of protein supplementation was assessed and thereafter the effect of protein supplementation during concomitant resistance exercise training compared with a control condition (**Table 3**) (20, 21, 33-66). Eleven studies exclusively included male subjects, whereas in 7 studies, female subjects were exclusively included. Thirteen studies included both sexes. The average habitual protein intake was below the protein recommendation of 0.8 g/kg/d in 1 study (61) (Table 3). Protein intervention strategies were heterogeneous and included 1) protein supplements (i.e. whey or (milk) protein, n = 11 or essential amino acids, n = 6), 2) multi-ingredient-nutrient supplements with protein and other nutrients such as micronutrients or omega fatty acids (n = 6), 3) food products with high protein content (i.e. ricotta, lean red meat, dairy, or soy) (n = 3) or 4) a diet with a high protein intake compared with a diet with a low protein intake (n = 5) (Table 3). With specific reference to the type of resistance exercise training performed, 17 studies performed whole-body resistance exercise training for 2-3 d/wk and 3 studies performed leg exercise for 3 d/wk. The intensity of the exercise training ranged between 50% and 85% of 1 repetition maximum (RM) (**Table 4**).

Table 2. Quality of included studies, based on Downs and Black checklist (26)

Authors (ref)	Reporting (maximum score = 11)	External validity (maximum score = 3)	Bias (maximum score = 7)	Confounding (maximum score = 6)	Total score (maximum score = 27)	Quality as per cutoff described
Aleman-Mateo et al. (35)	10	3	5	6	24	Good
Arnarson et al. (36)	8	3	7	6	24	Good
Bell et al. (34)	8	2	6	5	21	Good
Bemben et al. (37)	5	1	7	5	18	Moderate
Campbell et al. (38)	6	1	7	4	18	Moderate
Campbell et al. (39)	5	1	7	4	17	Moderate
Candow et al. (20)	5	1	7	3	16	Moderate
Carter et al. (40)	6	1	7	6	20	Good
Castaneda et al. (41)	8	0	5	4	17	Moderate
Chanet et al. (42)	11	2	7	6	26	Excellent
Daly et al. (43)	9	2	3	4	18	Moderate
Dillon et al. (44)	7	1	6	4	18	Moderate
Eliot et al. (45)	6	1	6	4	17	Moderate
Farnfield et al. (46)	6	1	6	3	16	Moderate
Godard et al. (47)	7	1	4	3	15	Moderate
Holm et al. (48)	6	3	7	4	20	Good
Iglay et al. (49)	7	2	5	4	18	Moderate
Iglay et al. (50)	8	2	4	3	17	Moderate
Ispoglou et al. (51)	4	2	7	2	15	Moderate
Kawada et al. (52)	6	1	6	2	15	Moderate
Kerstetter et al. (53)	9	2	7	5	23	Good
Kukuljan et al. (33)	10	2	4	4	20	Good
Leenders et al. (54)	5	1	6	5	17	Moderate
Markofski et al. (55)	8	1	7	5	21	Good
Meredith et al. (56)	7	1	5	3	16	Moderate
Mitchell et al. (57)	7	1	5	3	16	Moderate
Mitchell et al. (58)	10	3	5	6	24	Excellent
Nabuco et al. (59)	8	3	6	6	23	Good
Norton et al. (60)	9	2	5	5	21	Good
Rossato et al. (61)	10	1	5	3	19	Moderate
Scognamiglio et al. (62)	9	2	7	5	23	Good
Seino et al. (63)	11	3	6	6	26	Excellent
Thomson et al. (64)	8	3	6	5	22	Good
Torres et al. (65)	10	2	4	4	20	Good
Verdijk et al. (21)	8	1	7	5	21	Good
Zhu et al. (66)	9	3	5	6	23	Good

Table 3. Overview of the characteristics of the included studies (*n* = 36). Studies in orange are RCTs with resistance exercise training.

Authors (ref)	Group	*n*, M/F	Age, y	BMI, kg/m²	Duration, wk	Habitual protein intake, g/kg/d	Protein and placebo supplementation				
							Type of protein	Amount, g/d	Placebo type	Iso caloric	Timing
Aleman-Mateo et al. (35)	Prot	49 (25/24)	70.8 ± 7.6	26.9 ± 3.3	12	NR	Ricotta	18			B, L, D
	Con	49 (24/25)	69.6 ± 6.4	27.3 ± 3.8		NR			NP	NP	-
Arnarson et al. (36)	Prot	83	73.3 ± 6.0	28.1 ± 4.4	12	1.00 ± 0.26	Whey	20 (3d/wk)			AE
	Con	78	74.6 ± 5.8	29.4 ± 4.8		0.92 ± 0.30			CHO	Y	AE
Bell et al. (34)	Prot	25 (25/0)	71 (1)	28.9 (0.8)	6 excl. training + 12 incl. training	1.1 (0.3)	Whey (+ creatine + calcium + vit D + n-3 PUFA)	30			B, BBT
	Con	24 (24/0)	74 (1)	28.1 (0.7)		1.2 (0.3)			CHO	NR	B, BBT
Bemben et al. + Carter et al. + Eliot et al. (37, 40, 45)	Prot	11 (11/0)	58.2 (2.0)	28.6‡	14	0.95 ± 0.5	Whey	35 (3d/wk)			AE
	Con	10 (10/0)	56.1 (1.4)	31.3‡		0.94 ± 0.5			CHO	N	AE
Campbell et al. + Campbell, et al. (38, 39)	Prot	6 (5/1)	64 (2)	26.5‡	14	NR	Lactovovegetarian diet incl. milk-based beverages	125‡*			NA
	Con	6 (3/3)	66 (4)	25.5‡		NR	Lactovovegetarian diet incl. milk-based beverages	62‡*	NA	N	NA
Candow et al. (20)	Prot1	9 (9/0)	63.3 (1.1)	28.2‡	12	NR	Protein	26.3‡ (3d/wk)			BE
	Prot2	10 (10/0)	66.5 (1.7)	28.5‡		NR	Protein	25.6‡ (3d/wk)			AE
	Con	10 (10/0)	64.6 (1.3)	29.1‡		NR			CHO	NR	BE, AE

Authors (ref)	Group	*n*, M/F	Age, y	BMI, kg/m²	Duration, wk	Habitual protein intake, g/kg/d	Protein and placebo supplementation				
							Type of protein	Amount, g/d	Placebo type	Iso caloric	Timing
Castaneda et al. (41)	Prot	6 (0/6)	72 (1)	26.4 ± 1.7	9	NR	Diet incl. milk-based beverages	60.6*			NA
	Con	6 (0/6)	71 (2)	27.3 ± 1.1		NR	Diet incl. milk-based beverages	31.7*	NA	N	NA
Chanet et al. (42)	Prot	12 (12/0)	70.3 ± 4.3	24.4 ± 3.3	6	1.3 ± 0.3	Whey (+ leucine + vit D)	20			BB
	Con	12 (12/0)	70.8 ± 3.5	25.1 ± 2.5		1.3 ± 0.2			NR	N	BB
Daly et al. + Torres et al. (43, 65)	Prot	53 (0/53)	72.1 ± 6.4	27.7 ± 3.9	16	1.07 ± 0.37	Lean red meat	45 (6d/wk)			6d/wk
	Con	47 (0/47)	73.6 ± 7.7	27.6 ± 4.8		1.13 ± 0.40			CHO	N	6d/wk
Dillon et al. (44)	Prot	7 (0/7)	67 ± 1	26.9‡	12	NR	EAA	15			MAS
	Con	7 (0/7)	69 ± 3	26.8‡		NR			Lactose		MAS
Farnfield et al. (46)	Prot	9 (9/0)	68.1 ± 1.6	27.5 ± 0.8	12	1.5 ± 0.1	Whey	26.6 (3d/wk)			AER
	Con	9 (9/0)	67.4 ± 1.3	27.5 ± 1.0		1.1 ± 0.1			NR	N	AER
Godard et al. (47)	Prot	8 (8/0)	70.8 (1.5)	29.0‡	12	1.1‡	EAA + CHO	12			AER
	Con	9 (9/0)	72.1 (1.9)	24.3‡		1.1‡			NP	N	-
Holm et al. (48)	Prot	13 (0/13)	55 (1)	24 (1)	24	1.04‡	Whey (+ CHO + calcium + Vit D)	10 (3d/wk)			AE
	Con	16 (0/16)	55 (1)	27 (1)		0.89‡			CHO	N	AE
Iglay et al. + Iglay et al. (49, 50)	Prot	18 (8/10)	61 (2)	26.7 (0.9)	12	1.15 ± 0.17¥	Omnivorous diet	88*			NA
	Con	18 (9/9)	62 (2)	25.6 (0.8)		1.11 ± 0.34¥	Omnivorous diet	64*	NA	Y	NA
Ispoglou et al. (51)	Prot1	8 (3/5)	71.1 ± 2.7	26.6 ± 3.8	12	0.95±0.31¥	EAA	15 (20% LEU)			MEV
	Prot2	8 (4/4)	71.9 ± 3.0	26.7 ± 3.4		1.06±0.31¥	EAA	15 (40% LEU)			MEV
	Con	9 (4/5)	71.8 ± 2.7	26.3 ± 3.9		1.10±0.34¥			CHO	Y	MEV

Kawada et al. (52)	Prot1	10 (5/5)	65 ± 1	22.6‡	26	1.47‡	EAA	3			B
	Prot2	11 (3/8)	67 ± 3	22.8‡		1.33‡	EAA	6			B, D
	Con	8 (4/4)	70 ± 1	22.9‡		1.33‡			CHO	NR	B
Kerstetter et al. (53)	Prot	106 (17/89)	69.9 ± 6.1	26.1 ± 3.4	78	1.07 ± 0.03	Whey protein isolate	45			
	Con	102 (13/89)	70.5 ± 6.4	26.4 ± 4.0		1.06 ± 0.03			CHO	Y	NR
Kukuljan et al. (33)	Prot1	43 (43/0)	61.7 ± 7.6	27.4 ± 3.7	78	1.26 ± 0.32	Milk protein (with calcium and vit D)	13.2			B, R
	Prot2	43 (43/0)	61.7 ± 7.7	27.7 ± 3.3		1.23 ± 0.28	Milk protein (with calcium and vit D)	13.2			B, R
	Con1	44 (42/0)	60.7 ± 7.1	28.1 ± 3.3		1.32 ± 0.32			NP	NP	-
	Con2	42 (42/0)	59.9 ± 7.4	26.7 ± 2.9		1.33 ± 0.31			NP	NP	-
Leenders et al. (54)	Prot	27 (15/12)	M: 70 (1) F: 72 (2)	M: 27.2 (0.7) F: 24.2 (0.7)	24	M: 1.1 (0.1) F: 1.2 (0.1)	Milk protein	15			B
	Con	26 (14/12)	M: 70 (1) F: 69 (1)	M: 26.7 (0.6) F: 25.0 (0.4)					CHO	N	B
Markofski et al. (55)	Prot	13 (NR)	Range: 65-85	26.5 (1.0)	24	0.95 ± 0.04	EAA	15			BM
	Con	11 (NR)		28.0 (0.8)					CHO	NR	BM
Meredith et al. (56)	Prot	6 (6/0)	67.8 ± 1.1	24.8 ± 0.8	12	1.24‡	Protein (+ CHO + Fat + vit + min)	20			MAS
	Con	5 (5/0)	64.8 ± 2.3	25.4 ± 0.9		1.21‡			NP	NP	-
Mitchell et al. (57)	Prot	8 (8/0)	74.4 ± 5.4	26.9 ± 3.2	12	NR	Protein	14			B or AE
	Con	8 (8/0)				NR			CHO	Y	B or AE
Mitchell et al. (58)	Prot	14 (14/0)	73.7 ± 3.3	28.2 ± 3.3	10	1.1 ± 0.3	Omnivorous diet	136*			NA
	Con	15 (15/0)	74.7 ± 3.9	28.4 ± 5.1		1.2 ± 0.4	Omnivorous diet	74*		Y	NA
Nabuco et al. (59)	Prot1	24 (0/24)	67.5 ± 5.2	26.4 ± 5.2	12	0.92 ± 0.20	Whey	27.1 (3d/wk)			BE
	Prot2	23 (0/23)	66.2 ± 9.4	25.3 ± 5.4		0.94 ± 0.36	Whey	27.1 (3d/wk)			AE
	Con	23 (0/23)	66.5 ± 7.2	23.8 ± 3.7		0.95 ± 0.27			CHO	Y	BE, AE

Authors (ref)	Group	*n*, M/F	Age, y	BMI, kg/m²	Duration, wk	Habitual protein intake, g/kg/d	Protein and placebo supplementation				
							Type of protein	Amount, g/d	Placebo type	Iso caloric	Timing
Norton et al. (60)	Prot	31 (9/22)	62.2 ± 4.7	25.7 ± 3.1	24	1.2 ± 0.3	Milk protein	12‡			B, L
	Con	29 (5/24)	59.5 ± 5.8	25.9 ± 4.1		1.2 ± 0.3			CHO	Y	B, L
Rossato et al. (61)	Prot	11 (0/11)	63.4 ± 7.6	28.1 ± 5.5	10	0.82 ± 0.29	Omnivorous diet	77*			NA
	Con	12 (0/12)	63.0 ± 8.6	28.4 ± 6.0		0.78 ± 0.30	Omnivorous diet	59*	NA	Y	NA
Scognamiglio et al. (62)	Prot	48 (20/28)	74 ± 6	27.4 ± 3.6	12	NR	AA (CHO)	36			MANS
	Con	47(22/25)	74 ± 5	27.3 ± 3.7		NR			CHO	N	MANS
Seino et al. (63)	Prot	40 (6/34)	73.4 ± 4.3	22.9 ± 2.9	12	1.39 ± 0.36	Milk protein (micronutrients)	10.5			L
	Con	40 (7/33)	73.7 ± 4.3	22.9 ± 2.2		1.28 ± 0.26			NA	NA	NA
Thomson et al. (64)	Prot1	54 (25/29)	61.3 ± 6.9	27.7 ± 3.9	12	1.06 ± 0.10	Dairy	27			BLD, AE
	Prot2	64 (29/35)	61.7 ± 8.3	27.5 ± 3.7		1.08 ± 0.09	Soy	27			BLD, AE
	Con	61 (27/34)	61.5 ± 6.9	27.6 ± 3.3		1.02 ± 0.05			CHO	Y	BLD, AE
Verdijk t al. (21)	Prot	13 (13/0)	72 ± 2	26.5 ± 1.0	12	1.1 ± 0.1	Casein hydrolysate	20 (3d/wk)			BE, AE
	Con	13 (13/0)	72 ± 2	27.4 ± 1.1		1.1 ± 0.1			NR	NR	BE, AE
Zhu et al. (66)	Prot	101 (0/101)	74.2 ± 2.8	26.1 ± 3.8	104	1.2 ± 0.3	Milk protein	30			BB
	Con	95 (0/95)	74.3 ± 2.6	27.2 ± 4.0		1.1 ± 0.3			CHO	Y	BB

Data are means ± SDs or means (SEs).

AA, amino acids; AE, after exercise; AER, after exercise or at random; B, breakfast; BB, before breakfast; BBT, before bed time; BE, before exercise; BM, between meals; CHO, carbohydrate; Con, control; D, dinner; EAA, essential amino acids; excl., excluding; incl., including; L, lunch; LEU, leucine; MAS, morning and afternoon snack; MANS, morning, afternoon and night snack; MEV; supplement alongside food, morning and evening; N, no; NA, not applicable; NP, no placebo; NR, not reported; Prot, protein; R, random; ref, reference; vit D, vitamin D; Y, yes.

‡ Calculated from average weight (and height).

*Total daily protein intake.

¥ Requested data from authors.

Table 4. Overview of the resistance exercise training characteristics of the included studies with a resistance exercise intervention

		Training			
Authors	**Habitual exercise**	**d/wk**	**Type of training**	**RT intensity**	**Training volume**
Arnarson et al. (36)	82% regular PA, 67% ≥ 30 min/d	3	WBR	75-80% 1RM	3 sets * 6-8 reps
Bell et al. (34)	NR	3	WBR	65% - 80% 1RM	3 sets * 10-12 / 6-8 reps
Bemben et al. + Carter et al. + Eliot et al. (37, 40, 45)	NR	3	WBR	80% 1RM	3 sets * 8 reps
Campbell et al. + Campbell et al. (38, 39)	NR	3	WBR	80% 1RM	2-3 sets * 8-12 reps
Candow et al. (20)	NR	3	WBR	70% 1RM	3 sets * 10 reps
Daly et al. + Torres et al. (43, 65)	Prot: 9.3 ± 5.6 h/wk Con: 8.1 ± 4.0 h/wk	2	WBR	14-16 Borg scale	3 sets * 8-12 reps
Farnfield et al. (46)	NR	3	WBR	50-80% 1RM	2 sets
Godard et al. (47)	NR	3	Knee extension	80% 1RM	3 sets * 10 volitional exhaustion reps
Holm et al. (48)	Prot: 2 persons ran 1x per wk Con: 3 persons ran 1x per wk	2-3	WBR	10-20 rep max	3-5 sets * 8-15 reps
Iglay et al. + Iglay et al. (48)	Prot: 55 ± 7 units/wk Con: 57 ± 7 units/wk	3	WBR	80% 1RM	3 sets * 8 volitional exhaustion reps
Kawada et al. (52)	> 23 exercises/wk (3 MET*20 min)	2	WBR	30% 1RM	20-30 reps * 2 sets + 10 min standing cycling at 100 Watt
Kukuljan et al. (33)	Prot1: 3.7 ± 3.9 h/wk Prot2: 3.3 ± 3.8 h/wk Con1: 3.6 ± 3.4 h/wk Con2: 3.4 ± 4.1 h/wk	3	WBR	50-85% 1RM	2-3 sets * 8-12 reps
Leenders et al. (54)	M: 1.48 ± 0.19 MET-h/d F: 1.44 ± 0.15 MET-h/d	3	WBR	60-80% 1RM	3-4 sets * 8-15 reps
Meredith et al. (56)	NR	3	Upper leg	80% 1RM	3 sets * 8 reps
Mitchell et al. (57)	NR	3	WBR	75% 1RM	3-4 sets
Nabuco et al. (59)	NR	3	WBR	60-70% 1RM	3 sets * 8-12 reps
Rossato et al. (61)	NR	3	WBR	70% 1RM	1-6 sets * 8-12 reps

		Training			
Authors	**Habitual exercise**	**d/wk**	**Type of training**	**RT intensity**	**Training volume**
Seino et al. (63)	Prot: 61.7 ± 31.3 MET-h/wk Con: 66.4 ± 44.4	2	WBR	NA	2 sets * 20 reps
Thomson et al. (64)	NR	3	WBR	8 rep max	1-3 sets * 8-20 reps
Verdijk et al. (21)	NR	3	Leg	60-80% 1RM	4 sets * 8-15 reps

Data depicted as mean ± standard deviation. *Significantly different from control group. MET, Metabolic Equivalent of Task; NA, not applicable; PA, physical activity; RM, repetition maximum; reps, repetitions; RT, resistance exercise training; WBR, whole-body resistance exercise training.

Effect of protein supplementation

Lean body mass

Ten studies assessed the effect of protein supplementation compared with a control condition on lean body mass with the use of DXA. Protein supplementation was not associated with greater changes in lean body mass compared with the control condition (SMD = 0.11; 95% CI: -0.06, 0.28, *P* = 0.19) with no heterogeneity (**Figure 2**) after 41 ± 32 wk.

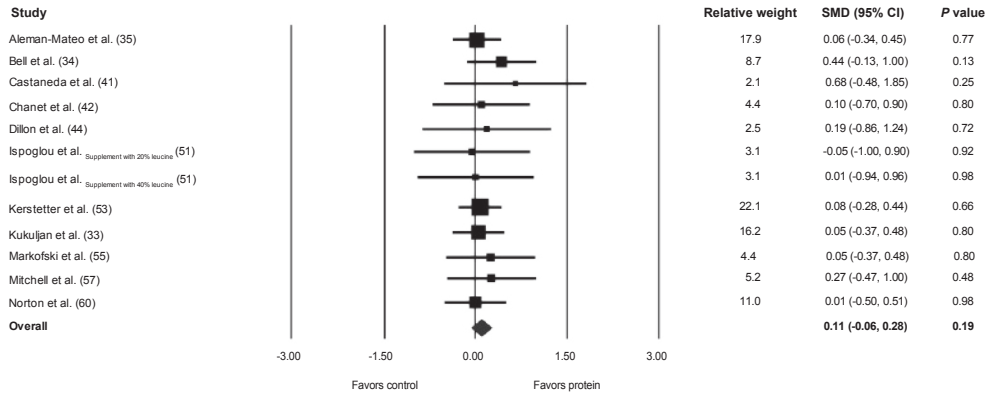


Figure 2. Forest plots of the standardized mean difference (SMD) of protein supplementation compared with a control condition on total lean body mass.

For each trial, the black square represents the point estimate of the intervention effect. The horizontal line joins the lower and upper limits of the 95% CI of this effect. The area of the black square represents the relative weight of the study in the meta-analysis. The black diamond represents the pooled estimate result (SMD) of the random-effects meta-analysis. No heterogeneity was present (Cochran's Q = 2.92; I^2 = 0.0%, *P* = 0.99).

Muscle strength

Seven out of 9 studies assessing the effect of protein supplementation on upper body strength focused on handgrip strength. Handgrip strength tended to increase more after 47 ± 45 wk of protein supplementation compared with the control condition (SMD: 0.58; 95%CI: -0.08, 1.24, *P* = 0.08) with significant heterogeneity (**Figure 3A**). No differences in the changes in lower extremity strength were observed between protein supplementation and control conditions after 90 ± 20 wk (SMD: 0.03; 95% CI: -0.20, 0.27, *P* = 0.78) (**Figure 3B**), and heterogeneity was absent.

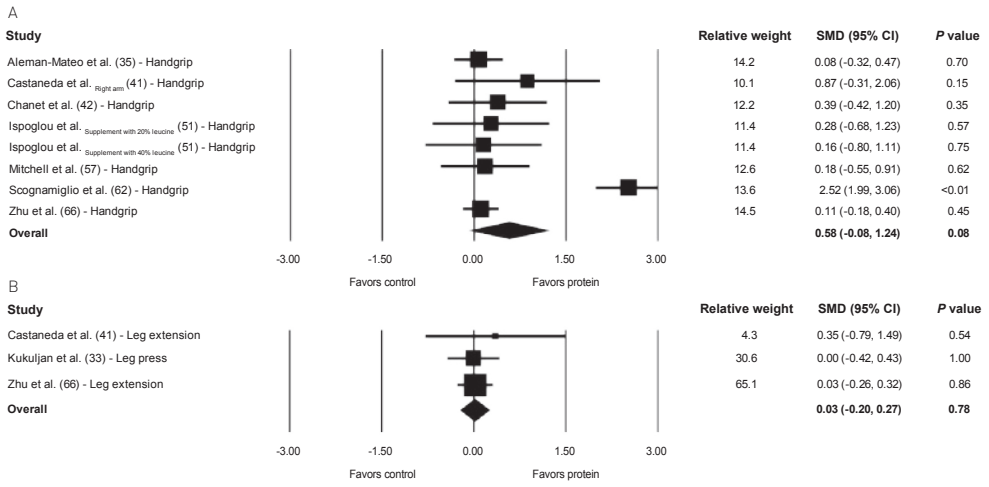


Figure 3. Forest plots of the standardized mean difference (SMD) of protein supplementation compared with a control condition on upper body (A) and lower extremity (B) muscle strength.

For each trial, the black square represents the point estimate of the intervention effect. The horizontal line joins the lower and upper limits of the 95% CI of this effect. The area of the black square represents the relative weight of the study in the meta-analysis. The black diamond represents the pooled estimate result (SMD) of the random-effects meta-analysis. Whereas significant heterogeneity was present (Cochran's Q = 67.0; I^2 = 89.6%, *P* < 0.001) in the analysis of the upper body (A), no heterogeneity was present (Cochran's Q = 0.33; I^2 = 0.0%, *P* = 0.85) in the analysis of the lower extremity (B).

Physical performance

Gait speed tended to increase more after 31 ± 30 wk protein supplementation compared with control conditions (SMD: 0.41, 95% CI -0.04, 0.85, *P* = 0.08, with significant heterogeneity (**Figure 4A**). No difference in chair-rise time was observed after 58 ± 42 wk of protein supplementation compared with control conditions (SMD: 0.10; 95% CI: -0.08, 0.28, *P* = 0.26), with no heterogeneity present (**Figure 4B**).

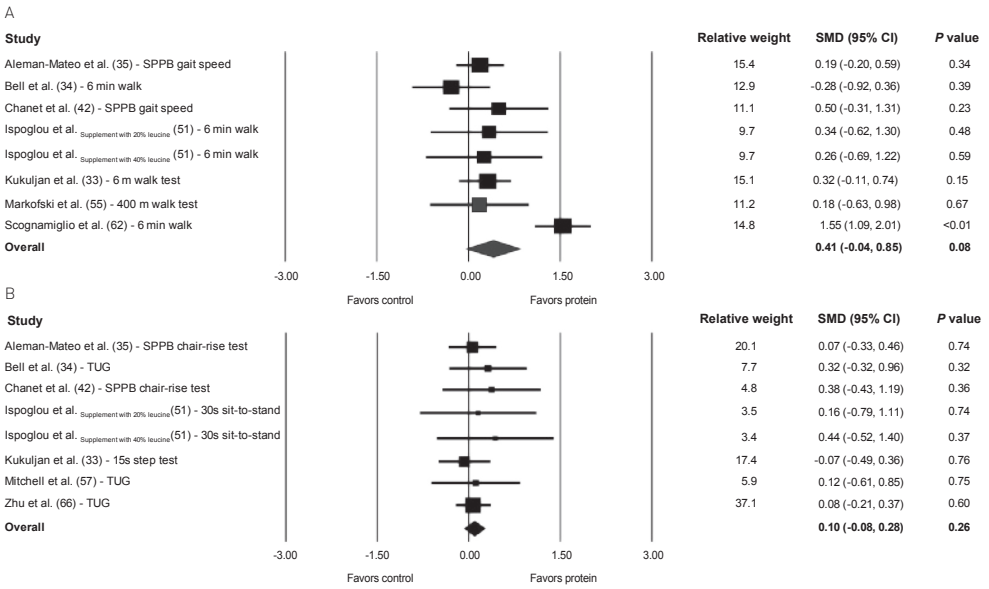


Figure 4. Forest plots of the standardized mean difference (SMD) of protein supplementation compared with a control condition on gait speed (A) and chair-rise performance (B).

For each trial, the black square represents the point estimate of the intervention effect. The horizontal line joins the lower and upper limits of the 95% CI of this effect. The area of the black square represents the relative weight of the study in the meta-analysis. The black diamond represents the pooled estimate result (SMD) of the random-effects meta-analysis. Whereas significant heterogeneity was present (Cochran's Q = 29.65; I^2 = 76.4%, P < 0.001) in the analysis of gait speed (A), no heterogeneity was present (Cochran's Q = 2.06; I^2 = 0.0%, P = 0.96) in the analysis of chair-rise performance (B). SPPB, Short Physical Performance Battery; TUG, Timed Up-and-Go test.

Effect of protein supplementation during resistance exercise training

Lean body mass

The additional effect of protein supplementation during concomitant resistance exercise training on lean body mass was assessed in 15 studies of which 11 studies used DXA (21, 33, 34, 36, 37, 40, 43, 45, 48, 54, 56, 61, 63, 64), 2 studies hydrostatic weighing (deuterium oxide dilution) (38, 39, 49, 50), 1 study measured with the Bod Pod (20), and another study determined body composition with hydro densitometry (underwater weighing) (56). Three of these included study arms reported fat-free mass rather than lean body mass (37, 39, 56, 67). Protein supplementation during resistance exercise training resulted in no significant larger effect on lean body mass compared with controls receiving solely resistance exercise training after 23 ± 25 wk (SMD: 0.08; 95% CI: -0.06, 0.21, P = 0.29) (**Figure 5**), with no heterogeneity present.

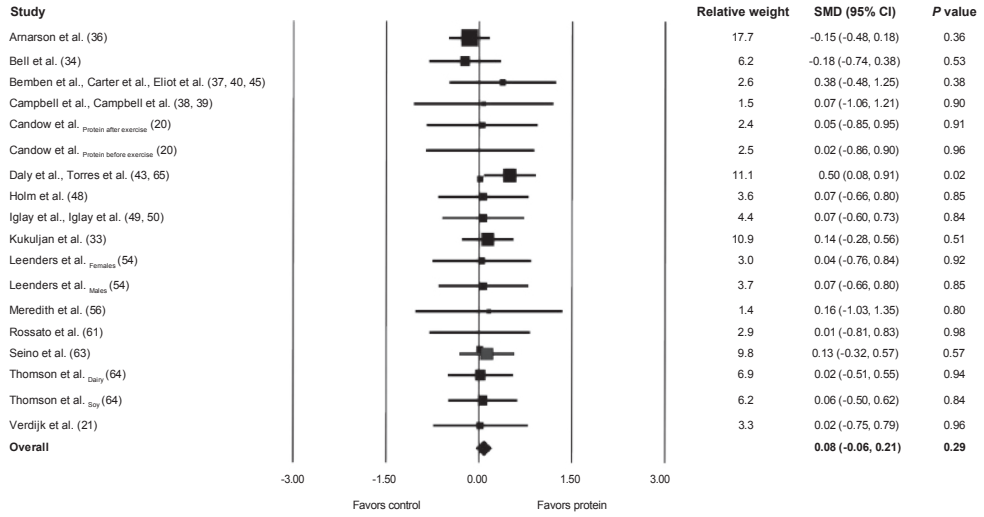


Figure 5. Forest plots of the standardized mean difference (SMD) of protein supplementation during resistance exercise training compared with a control condition during resistance exercise training on total lean body mass.

For each trial, the black square represents the point estimate of the intervention effect. The horizontal line joins the lower and upper limits of the 95% CI of this effect. The area of the black square represents the relative weight of the study in the meta-analysis. The black diamond represents the pooled estimate result (SMD) of the random-effects meta-analysis. No heterogeneity was present (Cochran's Q = 7.32; I^2 = 0.0%, *P* = 0.98).

Muscle cross-sectional area

Protein supplementation on top of resistance exercise was not associated with greater changes in thigh muscle cross-sectional area (SMD: 0.09; 95% CI: -0.23, 0.42, *P* = 0.57) compared with the control condition after 16 ± 6 wk with no heterogeneity present (**Figure 6**).

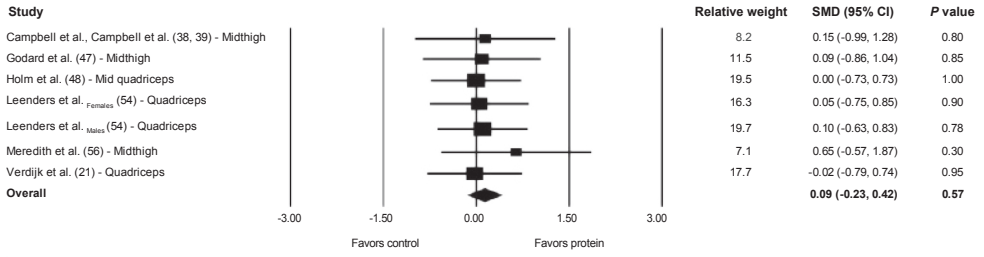


Figure 6. Forest plots of the standardized mean difference (SMD) of protein supplementation during resistance exercise training compared with a control condition during resistance exercise training on thigh muscle cross-sectional area.

For each trial, the black square represents the point estimate of the intervention effect. The horizontal line joins the lower and upper limits of the 95% CI of this effect. The area of the black square represents the relative weight of the study in the meta-analysis. The black diamond represents the pooled estimate result (SMD) of the random-effects meta-analysis. No heterogeneity was present (Cochran's Q = 0.97; I^2 = 0.0%, *P* = 0.99).

Muscle strength

Protein supplementation for 30 ± 31 wk did not yield greater improvement of upper body strength during resistance exercise training than the control condition receiving solely resistance exercise training (SMD: 0.11; 95% CI: -0.07, 0.29, *P* = 0.23) (**Figure 7A**), with no heterogeneity present. No significant differences for lower extremity muscle strength were found between protein supplementation and control conditions after 24 ± 24 wk of resistance exercise training with no heterogeneity present (**Figure 7B;** SMD: 0.10; 95% CI: -0.06, 0.27, *P* = 0.22).

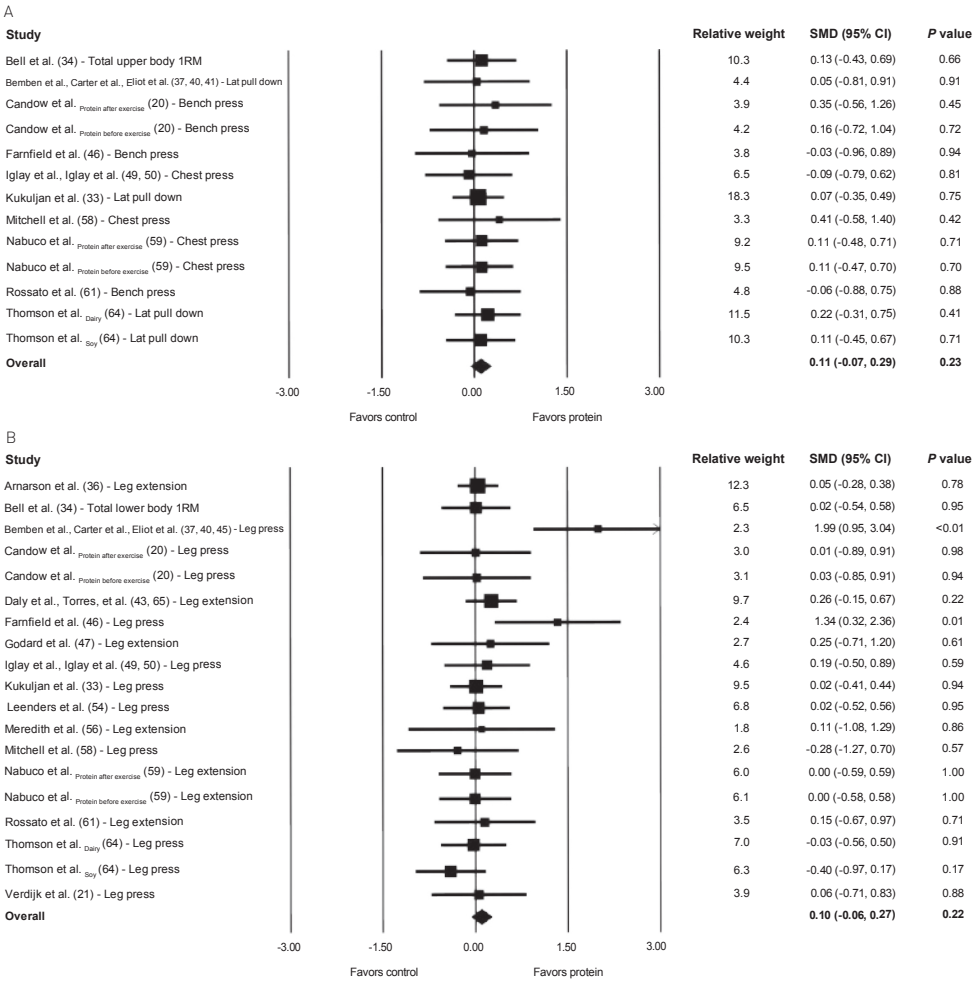


Figure 7. Forest plots of the standardized mean difference (SMD) of protein supplementation during resistance exercise training *versus* a control condition during resistance exercise training on upper body (A) and lower extremity (B) muscle strength.

For each trial, the black square represents the point estimate of the intervention effect. The horizontal line joins the lower and upper limits of the 95% CI of this effect. The area of the black square represents the relative weight of the study in the meta-analysis. The black diamond represents the pooled estimate result (SMD) of the random-effects meta-analysis. No heterogeneity was present in both analyses (Cochran's Q = 1.43, I^2 = 0.0%, *P* = 1.00 and Cochran's Q = 23.49; I^2 = 23.4%, *P* = 0.17, respectively).

Physical performance

Gait speed tended to increase more with protein supplementation compared with the control condition, whereas no differences were observed in chair-rise time after 26 ± 26 wk and 28 ± 26 wk of resistance exercise, respectively (gait speed SMD: 0.13; 95% CI: -0.03, 0.28, *P* = 0.10; chair-rise SMD: -0.01; 95% CI: -0.16, 0.17, *P* = 0.95), with no heterogeneity present (**Figure 8A, B**).

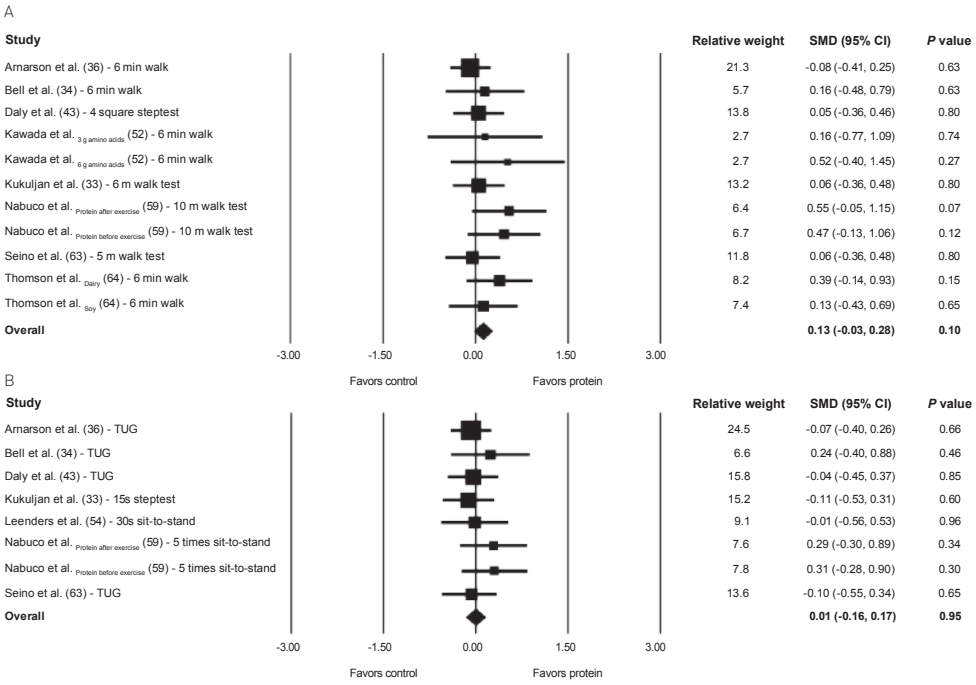


Figure 8. Forest plots of the standardized mean difference (SMD) of protein supplementation during resistance exercise training compared with a control condition during resistance exercise training gait speed (A) and chair-rise performance (B).

For each trial, the black square represents the point estimate of the intervention effect. The horizontal line joins the lower and upper limits of the 95% CI of this effect. The area of the black square represents the relative weight of the study in the meta-analysis. The black diamond represents the pooled estimate result (SMD) of the random-effects meta-analysis. No heterogeneity was present in both analyses (Cochran's Q = 7.16; I^2 = 0.0%, *P* = 0.71 and Cochran's Q = 3.24; I^2 = 0.0%, *P* = 0.86, respectively). TUG, Timed Up-and-Go test.

Publication bias and sensitivity analyses

Considerable symmetry was observed on examining the individual funnel plots of SE by Hedge's g and the ''trim and fill'' algorithm, which implied there was no publication bias for the analyses (**Supplemental Figures 1 – 7**). The ''remove-one'' analyses revealed that no single study significantly alters the magnitude and direction for the analyses of lean body mass, muscle strength and physical performance in the analyses assessing the effect of protein supplementation compared with controls and in the analyses that assessed the effect of protein supplementation compared with controls during concomitant resistance exercise training.

DISCUSSION

The present work is the first meta-analysis to assess the effect of protein supplementation on lean body mass, muscle strength, and physical performance in nonfrail community-dwelling older adults. We found no beneficial effects of protein supplementation on lean body mass, muscle strength, and chair-rise ability. We did find a tendency of a larger increase in handgrip strength and gait speed after protein supplementation compared with the control condition. In addition, the effect of additional protein supplementation during resistance exercise training on lean body mass, muscle thigh cross-sectional area, muscle strength, and chair-rise ability was not superior to resistance exercise training only in nonfrail older adults, whereas we observed a borderline significant larger increase in gait speed with protein supplementation compared with the control condition during concomitant resistance exercise training. These observations are contradictory to previously reported outcomes, and suggest that protein supplementation may only exert beneficial effects in specific groups of older adults.

Effect of protein supplementation

We found no effects of protein supplementation on lean body mass, muscle strength and/or physical performance in nonfrail community-dwelling older adults, except for a tendency of a larger increase in handgrip strength and gait speed after protein supplementation compared with the control condition. These borderline significant findings, however, were hampered by significant heterogeneity, which was caused by the pronounced positive findings of a single study (62). Participants of this study in healthy elderly had baseline grip strength and gait speed scores far below recommendations (68), which allowed for large improvements in handgrip strength and gait speed. Furthermore, participants received a very high dose of supplementation: 35g of amino acids. Thus, the findings of Scognamiglio et al. (62) should be interpreted with care, as they may not be representative of the effects of protein intake in general nonfrail community-dwelling older adults.

The absent beneficial effects of protein supplementation in our meta-analysis are in contrast with RCTs that found a positive effect of protein supplementation in frail older adults (69-73). It has been suggested that the beneficial effects of protein supplementation may be different between frail and nonfrail older adults (74), because a higher chronic inflammatory activity in frail elderly may cause an attenuated muscle protein metabolism compared with nonfrail older adults (8, 75, 76). Therefore, protein supplementation may only exert beneficial adaptations in frail older adults.

There are several other explanations for the lack of beneficial effects of additional protein on muscle characteristics in the here-presented population of nonfrail community-dwelling older adults. First, the mean habitual protein intake of study participants was higher than the Recommended Dietary Allowance of 0.8 g/kg/d. Therefore, protein intake might have already been sufficient to counteract the age-related anabolic resistance (77) and additional protein supplementation beyond the Recommended Dietary Allowance may have no additional effect on body and muscle characteristics. Given the curvilinear and saturable dose-response relation between protein intake and muscle protein synthesis (8), it may be possible that nonfrail older individuals reach a plateau phase when a protein intake >0.8 g/kg/d is achieved. Second, the protein supplementation protocol could have been suboptimal in some studies. Older individuals require higher per-meal protein doses to achieve similar rates of muscle protein synthesis compared with younger individuals (78), especially because postprandial muscle protein synthesis is blunted in older adults (79, 80). Incorporating doses of 25-30 g protein in the diet of older adults has been suggested as a promising strategy to counteract the attenuated postprandial muscle protein synthesis (81, 82). Detailed information on protein supplementation protocols was not provided in most of the studies, so we cannot exclude the possibility that suboptimal interventions may have affected outcomes of our meta-analysis.

Effect of protein supplementation during concomitant resistance exercise training

Studies have shown that resistance exercise induces a muscle protein synthetic response in the ageing muscle (83-85). Therefore, resistance exercise in combination with protein supplementation has been proposed to have synergistic effects on muscle characteristics in older adults (8, 86). We found no superior effect of protein supplementation in combination with resistance exercise training compared with resistance exercise training only on changes in lean body mass, thigh muscle cross-sectional area, muscle strength, and chair-rise ability in nonfrail older adults. Gait speed was borderline significantly more improved with protein supplementation compared with the control condition during concomitant resistance exercise training. These observations align with meta-analyses that assessed the effects of protein supplementation during concomitant resistance exercise training on muscle strength (14, 87), but are contradictory to changes found in lean body mass (14, 88, 89). This apparent discrepancy in lean body mass between our analysis and previous meta-analyses (14, 88, 89) might be partly

explained by the inclusion of studies with (pre-)frail participants (18, 19, 90) in these meta-analyses. The RCTs in those meta-analyses had a relatively large weight owing to the large sample size of (pre-)frail older adults and found a positive effect of protein supplementation (14, 88, 89). An alternative explanation for the discrepancy may be the very strong correlation coefficient of r=0.98 used (88), whereas we used a correlation factor of r=0.50 according to reported literature (29, 30). Indeed, our sensitivity analyses on our pooled dataset revealed that a significant beneficial effect of protein supplementation was achieved with a correlation factor $r \geq 0.98$, but not with lower correlation factors. A third explanation for the contradictory findings of protein intake on lean body mass changes may relate to the contribution of protein intake-induced change in lean organ mass. Animal studies have demonstrated hypertrophy of organs such as the kidney and liver in response to high-protein diets (91, 92), which may contribute to the increased lean body mass. However, the increase in organ mass only occurred with high protein levels (92). It is still unclear how these findings translate to humans because the hypertrophic effects of protein intake on human organs are currently unknown. Future studies assessing the distinct effects of protein intake on muscle mass compared with organ mass are therefore warranted. On the other hand, contradicting results might also be caused by differences in protein supplementation protocols between the RCTs. In our meta-analysis, there are distinct differences among RCTs in the timing of protein intake, and the type and amount of protein, but these factors are known to affect changes in body composition and/or muscle characteristics (93). Finally, habitual physical activity levels of participants are often not taken into account, yet these could influence the magnitude of the anabolic response (83-85). The lack of superior effects of protein supplementation during concomitant resistance exercise training in nonfrail community-dwelling older adults indicates that we should critically look at the current dogma that protein supplementation during resistance exercise training has additional beneficial effects for muscle characteristics among older adults in general, regardless of their frailty status, physical activity levels, or habitual protein intake. On the other hand, if additional protein is provided one should carefully assess which type, amount, and timing of the protein intake has the most beneficial effects.

Limitations

In our meta-analysis we included RCTs that used protein or essential amino acid supplements, protein-based multi-ingredient-nutrient supplements, food products with high protein content and diets with a high protein intake compared with diets with a low protein intake. The large variety in protein supplementation strategies resulted in a larger number of studies that could be included in our meta-analysis. However, it also induces more heterogeneity, which represents a potential limitation when interpreting the results of this meta-analysis. Significant heterogeneity was present in only 2 forest plots, assessing the effect of protein supplementation compared with control conditions on handgrip strength and gait speed. To correct for possible heterogeneity we adopted a random-effect approach and our sensitivity analyses indicated that

our results showed good robustness. Nevertheless, the small number of studies with similar protein supplementation protocols highlights the need for additional long-term studies that assess which amount, type, or timing of protein gives beneficial effects in a homogeneous population of nonfrail older adults to address whether protein supplementation can delay the onset of sarcopenia.

Conclusion

In conclusion, protein supplementation in nonfrail community-dwelling older adults does not lead to increases in lean body mass, thigh muscle cross-sectional area, muscle strength or physical performance compared with control conditions, nor does it exert superior effects when added to resistance exercise training. Habitual protein intakes of most study participants were already sufficient and protein interventions differed in relation to type of protein, amount and timing. Future research should focus on optimization of protein intake in nonfrail community-dwelling older adults with low habitual protein intake and assess whether these individuals could benefit from particular protein supplementation protocols.

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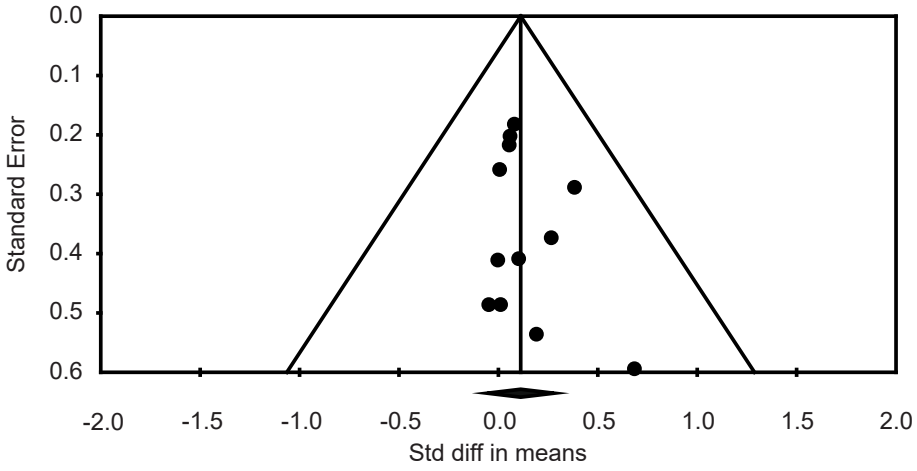
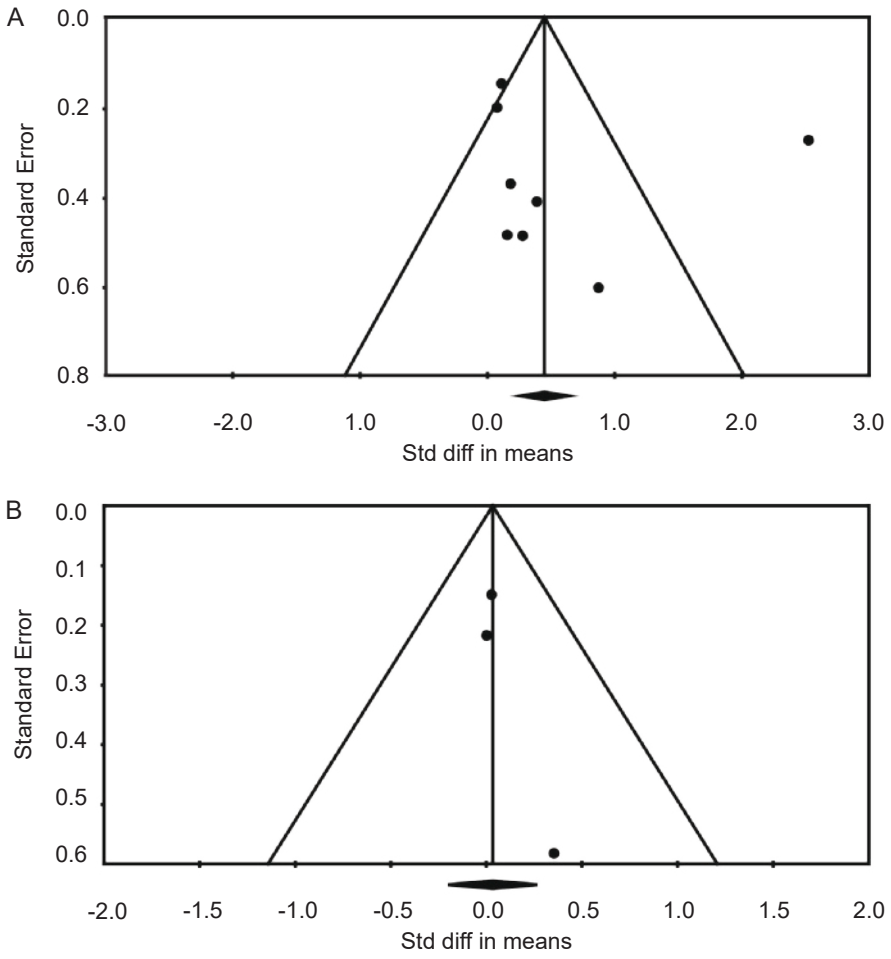
SUPPLEMENTAL MATERIAL

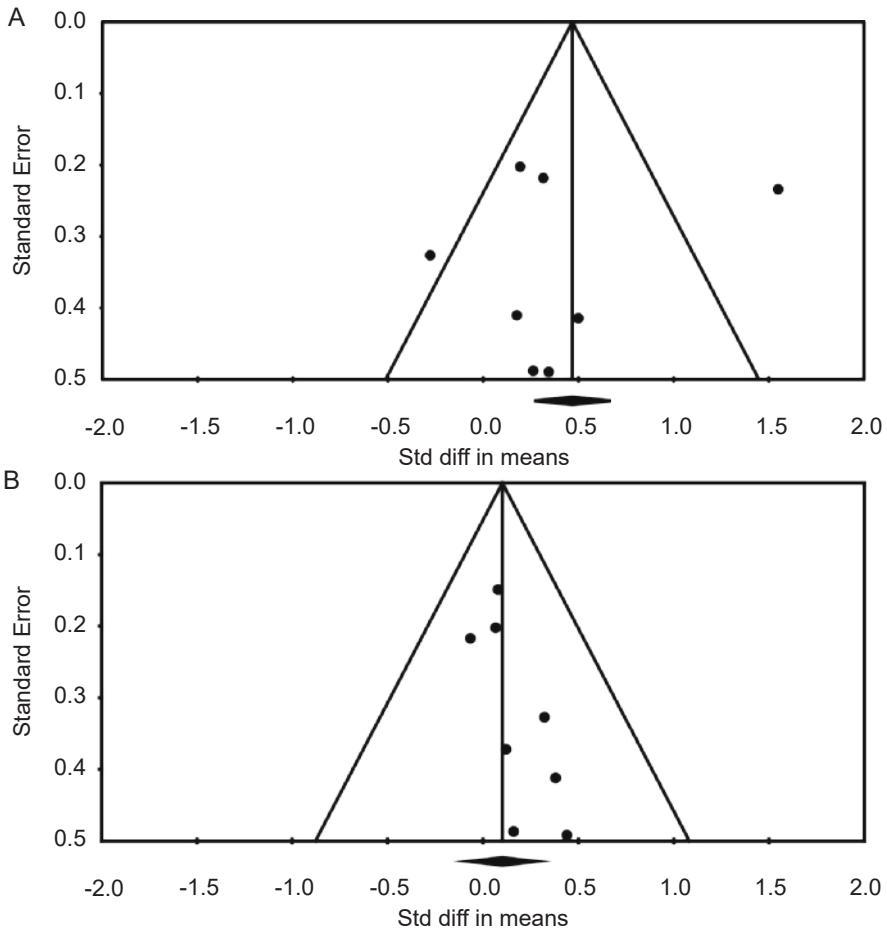


Supplemental Figure 1. Funnel plot of standard error by Hedge's g and the Trim 'n fill algorithm' of studies assessing the effect of protein supplementation on lean body mass.

The standardized difference in means is depicted against the standard error.

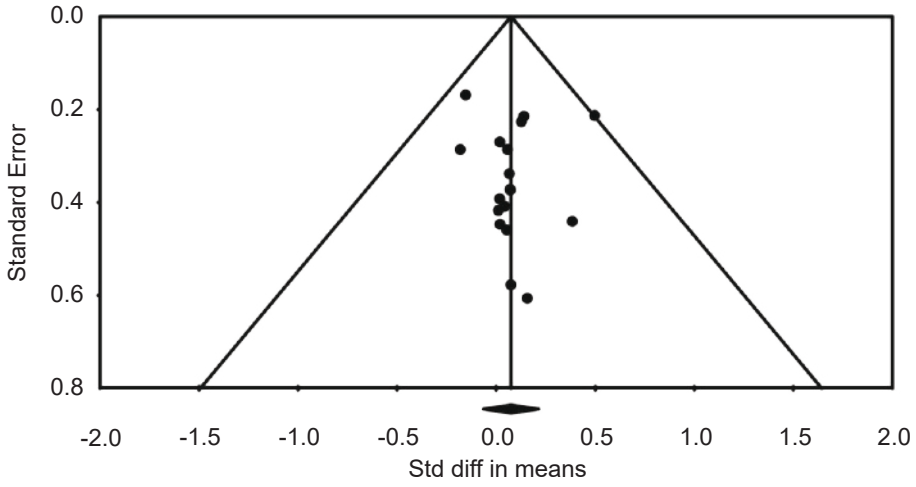


Supplemental Figure 2. Funnel plot of standard error by Hedge's g and the Trim 'n fill algorithm' of studies assessing the effect of protein supplementation on upper body (A) and lower extremity (B) muscle strength. The standardized difference in means is depicted against the standard error.



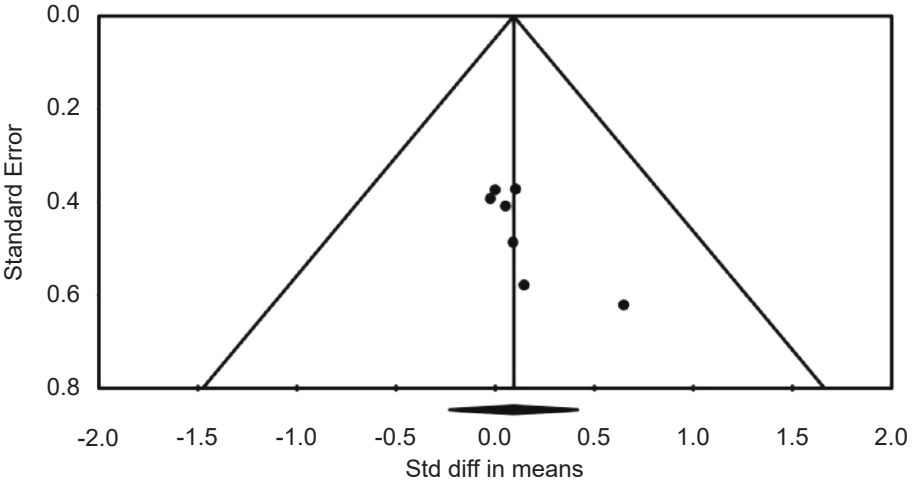
Supplemental Figure 3. Funnel plot of standard error by Hedge's g and the Trim 'n fill algorithm' of studies assessing the effect of protein supplementation on gait speed (A) and chair-rise performance (B).

The standardized difference in means is depicted against the standard error.



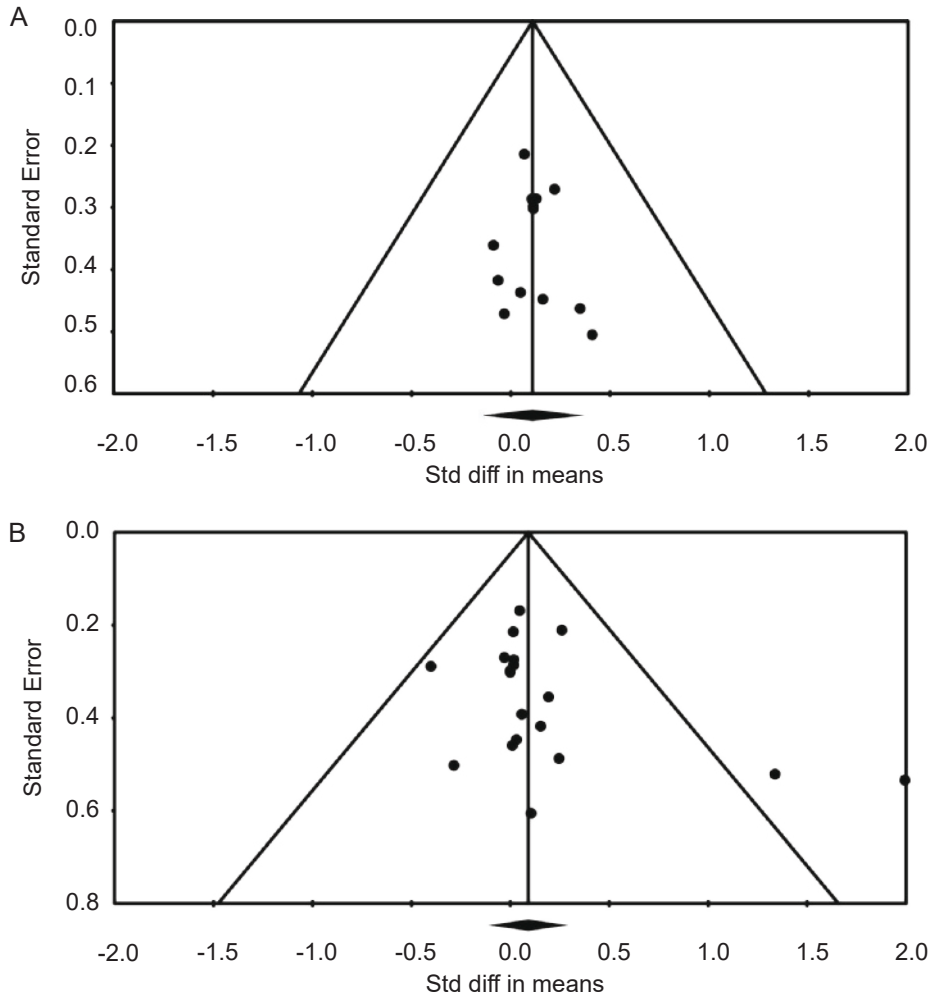
Supplemental Figure 4. Funnel plot of standard error by Hedge's g and the Trim 'n fill algorithm' of studies assessing the effect of protein supplementation during concomitant resistance exercise training on lean body mass.

The standardized difference in means is depicted against the standard error.



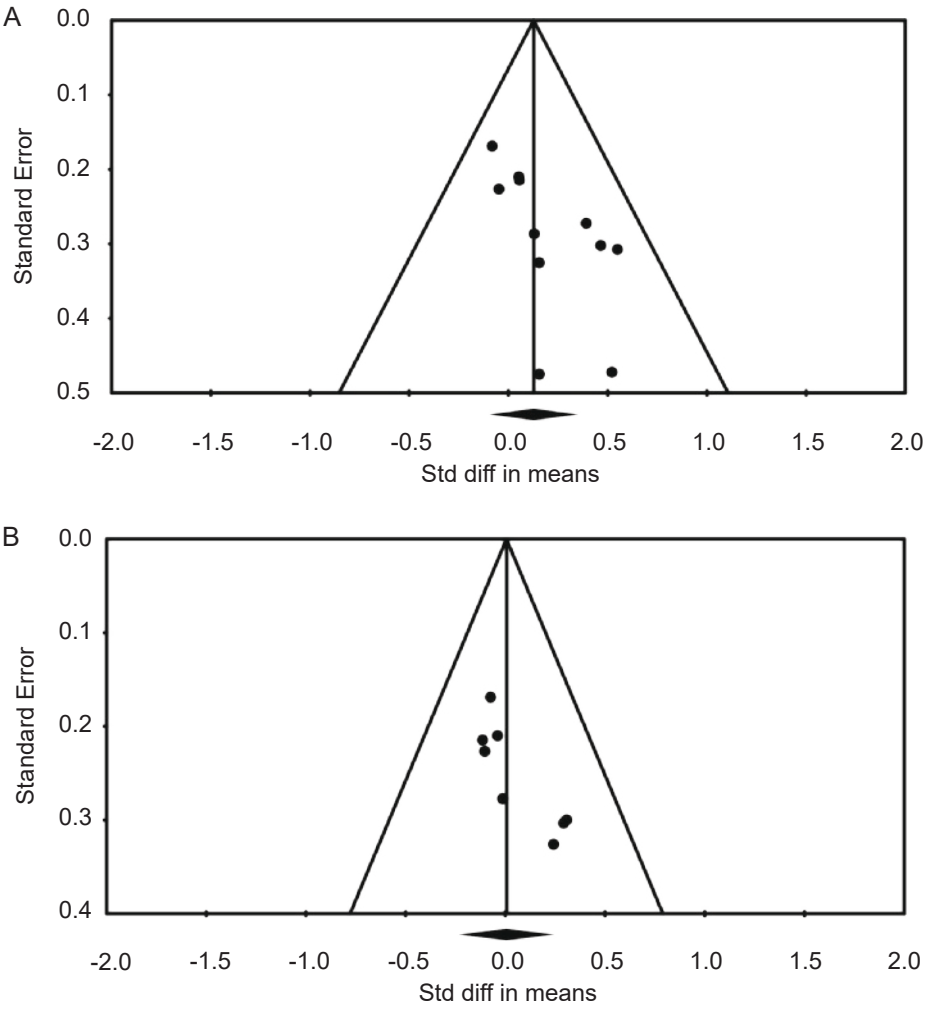
Supplemental Figure 5. Funnel plot of standard error by Hedge's g and the Trim 'n fill algorithm' of studies assessing the effect of protein supplementation during concomitant resistance exercise training on muscle cross-sectional area of the thigh.

The standardized difference in means is depicted against the standard error.



Supplemental Figure 6. Funnel plot of standard error by Hedge's g and the Trim 'n fill algorithm' of studies assessing the effect of protein supplementation during concomitant resistance exercise training on upper body (A) and lower extremity (B) muscle strength.

The standardized difference in means is depicted against the standard error.



Supplemental Figure 7. Funnel plot of standard error by Hedge's g and the Trim 'n fill algorithm' of studies assessing the effect of protein supplementation during concomitant resistance exercise training on gait speed (A) and chair-rise performance (B).

The standardized difference in means is depicted against the standard error.

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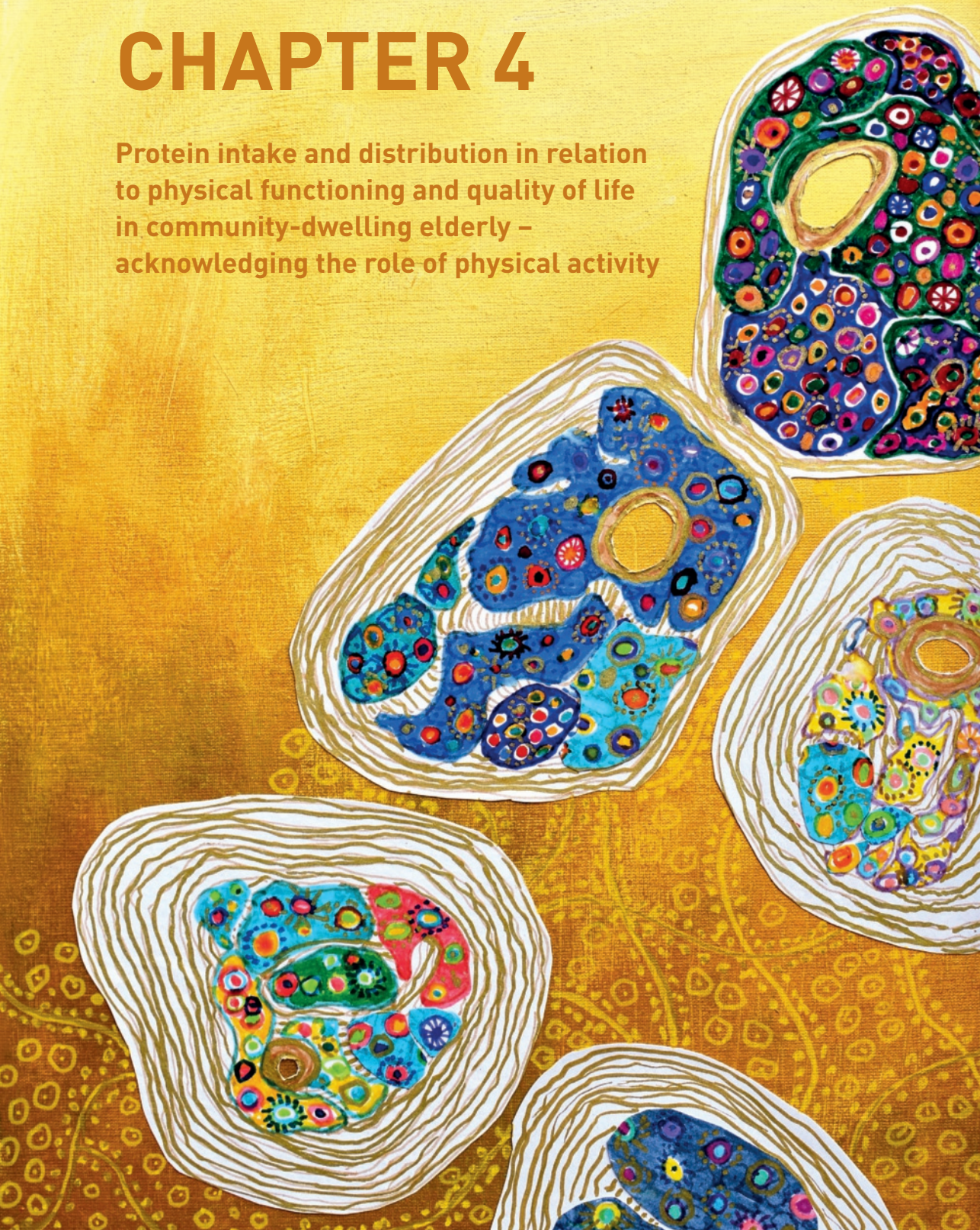
CHAPTER 4

Protein intake and distribution in relation to physical functioning and quality of life in community-dwelling elderly – acknowledging the role of physical activity



ABSTRACT

Increasing total protein intake and a spread protein intake distribution are potential strategies to attenuate sarcopenia related loss of physical function and quality of life. The aim of this cross-sectional study was to investigate whether protein intake and protein intake distribution are associated with muscle strength, physical function and quality of life in community-dwelling elderly with a wide range of physical activity. Dietary- and physical activity data were obtained from two studies (N=140, age 81 ± 6, 64% male), with outcome measures: physical functioning (Short Physical Performance Battery, comprising balance, gait speed and chair rise tests), handgrip strength and quality of life (EQ-5D-5L). Protein intake distribution was calculated for each participant as a coefficient of variance (CV = SD of grams of protein intake per main meal divided by the average total amount of proteins (grams) of the main meals). Based on the CV, participants were divided into tertiles and classified as spread, intermediate or pulse. The average total protein intake was 1.08 ± 0.29 g/kg/d. Total protein intake was not associated with outcome measures using multivariate regression analyses. Individuals with a spread protein diet during the main meals (CV <0.43) had higher gait speed compared to those with an intermediate diet (CV 0.43-0.62) (β = –0.42, P = 0.035), whereas a spread and pulse protein diet were not associated with SPPB total score, chair rise, grip strength and Quality-Adjusted Life Year (QALY). The interaction of higher physical activity and higher total protein intake was significantly associated with higher quality of life (β = 0.71, P = 0.049). While this interaction was not associated with SPPB or grip strength, the association with quality of life emphasizes the need for a higher total protein intake together with an active lifestyle in the elderly.

INTRODUCTION

Sarcopenia is defined as the age-related loss of muscle mass and muscle strength, resulting in impaired physical function [1] and loss of independence for daily life activities [2], which is associated with a decreased quality of life and an increased health care expenditure [3,4]. On average, 5-13% of elderly aged 60-70 years are affected by sarcopenia with prevalence increasing to 11-50% in elderly over the age of 80 years [5]. Therefore, it is important to identify strategies to counteract sarcopenia.

Sufficient protein intake is essential for muscle protein synthesis and the consequent preservation or improvement of muscle mass and strength [6]. Based on the age-related decline in protein utilization for muscle protein synthesis [7-9], Bauer *et al*. proposed a protein intake for elderly of 1.0-1.2 g protein per kilogram body weight per day (g/kg/d) [6], a dose which is well above the current recommendations of 0.8 g/kg/d for all adults [10,11].

Besides the amount of protein intake, the protein intake distribution might be associated with muscle mass and strength. Some studies support a pulse-feeding pattern in which a high protein meal might saturate the splanchnic sequestration leading to a higher availability of amino acids for muscle protein synthesis [12,13]. In contrast, several other studies reported an optimized muscle protein synthesis using a more continuous availability of amino acids in a spread-feeding pattern [14-18]. As yet, the current literature is not conclusive about the most efficient protein intake distribution for optimal muscle protein synthesis.

Another strategy to influence muscle strength as well as physical function in the elderly is to enhance physical activity [19,20]. In addition to the independent effect of physical activity on muscle strength, previous literature reported an exercise-induced increase in anabolic sensitivity to dietary protein for up to 24 hours after exercise [21]. It seems therefore of utmost importance to take physical activity of the individual into account when studying the effect of protein intake and distribution on muscle characteristics. Unfortunately most studies do not include physical activity in the equation [12,13,15].

Therefore, the aim of this study is to investigate whether protein intake and protein intake distribution are associated with muscle strength, physical function and quality of life in community-dwelling elderly people additionally accounting for the role of physical activity. We hypothesize that higher protein intake and a spread protein distribution are associated with improved strength and physical function, while adding physical activity to the equation will enlarge these effects.

METHODS

Study population

This study included data collected from 140 adults aged 65 years or older. Participants were from two studies, thus creating a wide range of physical activity. The first sample included participants of the Nijmegen Four Days Marches. These elderly people were approached via mail, and a total of 82 participants were included. To include a sample of less active elderly people, baseline data of participants from the ProMuscle in Practice study (n=58) were included. Participants were mainly recruited through local media outings, flyers, and home care providers. This trial was registered in the Netherlands Trial Register (NTR6038). Both studies were approved by a local Medical Ethical Committee (CMO registration number: 2007/148 and 16/12, respectively), conducted in accordance with the Declaration of Helsinki, and all participants gave written informed consent prior to participation.

Study design

In the present cross-sectional study, we measured protein intake, physical activity, muscle strength, physical functioning, and quality of life in 140 participants from two distinct studies. Eighty-two participants were recruited within the Four Days Marches study. Measurements were performed one or two days prior to the Four Days Marches, while the participants' habitual dietary intake was assessed one month later to make sure their intake was representative of a regular period of the year. The remaining 58 participants were recruited within the ProMuscle in Practice study, whereas all measurements were performed before participants started the intervention.

MEASUREMENTS

Protein intake

In the Four Days Marches study, daily dietary intake was assessed using two 24-hr recalls, a validated method for assessing the amount and distribution of protein intake [22]. The two recall days were randomized over the week with the restriction that no participant was assigned to two identical week days or to two weekend days. The 24hr recalls were performed face-to-face or by phone by trained dieticians. Portion sizes were documented in household measures, whereby frequently used household measures were subsequently quantified with standard portion sizes. In the ProMuscle in Practice study, dietary intake was assessed using three-day food records, which is another validated method to measure protein intake in the elderly [23]. Each participant was randomly assigned to two weekdays and one weekend day. Research dietitians gave oral and written instructions about completing the food record. After completion of the food record, a trained research dietitian visited the participants at home

to check the food record. During this home visit, the dietitian also weighed and measured a standardized selection of food items and household measures that were linked to protein intake. De Keyzer *et al.* [24] reported a fair strength of agreement between the 24hr recalls and the food records for protein intake, and concluded that group level intakes of protein did not differ [24]. Data were coded by trained dietitians and energy and macronutrient intake was calculated using Compl-eat, based on the Dutch food composition table (NEVO, 2013) [25]. The mean of the recorded days was calculated for total daily intake and intake per main meal (breakfast, lunch, dinner).

Evaluation of underreporting energy intake

Underreporting of energy intake (EI) of the participants was evaluated with Goldberg's method, which is based on the ratio of energy intake and basal metabolic rate (BMR) [26]. BMR was calculated using Schofield's equations, which is based on age, body weight, and height [27]. To set a Goldberg cut–off value to identify underreporting or overreporting, we assumed a within–subject variation in energy intake of 23%, a within–subject variation in estimated BMR of 8.5%, a physical activity level of 1.55, and a between–subject variation in physical activity level of 15% [26,28,29]. For participants ≥70 years, a Goldberg score of <0.89 was defined as underreporting and >2.66 as overreporting [29]. Analysis were performed with and without inclusion of participants that were under- or overreporting and when results were different, both were reported.

Physical activity

In the Four Days Marches study, physical activity was assessed by the Short Questionnaire to Assess Health enhancing physical activity (SQUASH), which is considered a valid and reliable method in the elderly [30]. This self-administered questionnaire estimates habitual level of physical activity during a normal week over the past month. In the ProMuscle in Practice study, physical activity was assessed using the LASA physical activity questionnaire (LAPAQ), which is another valid method to assess physical activity in elderly [31]. It measures physical activities performed in the past two weeks, and was completed together with a researcher.

Both questionnaires include walking, cycling, gardening, light and heavy household activities, and sports activities. Information was collected about type, duration and frequency of these activities. The intensity for each activity was determined based on activity intensity classification according to Ainsworth's Compendium of Physical Activities [32]. Total physical activity and activity-specific activity could be calculated in MET-hours per day (METhr/day) by multiplying the exercise time in hours with the accompanying MET score of the activity intensity [32].

Muscle strength

In both studies, muscle strength was measured by handgrip strength of the dominant hand. This was measured with a hydraulic, analogue hand dynamometer (Jamar, Jackson, MI, USA). For every participant the dynamometer was adjusted to their hand size. The participants were seated in a chair with the elbow flexed in a 90-degree angle position. Arm support by the chair was not allowed. Three measurements were performed with approximately 30 seconds rest between measurements. The maximum strength effort in kilograms was used for analysis.

Physical functioning

In both studies, physical function was assessed by the Short Physical Performance Battery (SPPB), which is considered a reliable and valid method in elderly [33]. The SPPB consists of three components: balance, gait speed, and chair rise ability. In the balance test, participants were asked to stand still for 10 s in three positions: feet side by side, feet in semi-tandem position, feet in tandem position. Gait speed is determined by the time necessary to complete a walk of 4 m at normal gait speed. The chair rise ability score was determined by the time necessary to rise out of a chair and sit down five times in a row, without the aid of the arms. For each component, a score of 0-4 points could be earned. A SPPB total score (0-12 points) was calculated by summing up the scores of the three tests, in which a higher score reflects a better physical function.

Quality of life

Quality of life was measured in both studies using the EQ-5D-5L questionnaire. This five-item questionnaire includes the domains mobility, self-care, usual activities, pain and discomfort, and anxiety/depression. Each question has five levels of functioning, ranging from no problems (1) to very severe problems (5). This questionnaire was used to calculate Quality-Adjusted Life Years (QALYs) [34]. Additionally, participants scored their current perceived health on a scale from 0 (worst imaginable health) to 100 (best imaginable health).

Background characteristics

Height and weight of each participant were measured and used to calculate BMI. Furthermore, additional questions about smoking, level of education, and use of (vitamin D) supplements were included in the questionnaire.

Statistical analysis

The statistical analyses were performed using SPSS 22 software (IBM SPSS Statistics for Windows, Version 22 IBM Corp., Armonk, NY, USA). All continuous variables were visually inspected and tested for normality with the Shapiro-Wilk test. Participant characteristics were displayed as means ± SDs or median (IQR) for parametric and non-parametric continuous variables respectively, and as counts with percentages for categorical variables. First,

participants were stratified into two groups based on protein intake with a cut-off 1.0 g/kg/d, and differences between these groups were tested with an independent samples t-test for parametric variables, Kruskal-Wallis test for non-parametric variables, or Chi-square test for categorical data. Second, protein intake distribution was calculated for each participant as a coefficient of variance (CV = SD of grams of protein intake per main meal divided by the average total amount of proteins (grams) of the main meals). Based on the CV, participants were divided into tertiles. A low CV represents less difference in protein intake between the meals, and therefore a more spread distribution, whereas a high CV represents a pulse-feeding distribution of protein intake. Differences between tertiles were tested using an ANOVA for parametric variables, a Kruskal-Wallis test for non-parametric variables and a Chi-square test for categorical data. Furthermore, the associations between protein intake, protein distribution and physical activity and physical function, muscle strength, and quality of life were analyzed in a multivariate linear regression (forced entry method, including confounders age, sex, BMI and protein source). Statistical significance was assumed at $P < 0.05$ (two–sided).

RESULTS

Descriptive characteristics

A total of 140 participants (90 males and 50 females) with a median age of 83 years (interquartile range (IQR): 77-84) were included in the analysis (**Table 1**). Body mass index (BMI) was 25.9 ± 2.7 kg/m^2 in males and 26.4 ± 4.7 kg/m^2 in females. Habitual energy intake was 2040 ± 370 kcal for males and 1754 ± 396 kcal for females. Based on the Goldberg-cutoff there were no participants overreporting and five participants (3.6%) underreporting their energy intake. The average total protein intake was 79 ± 19 g/d– or 1.08 ± 0.29 g/kg/d when adjusted for bodyweight– and 62 ± 9% was animal source protein. Thirty participants (21%) used vitamin D containing supplements. Total physical activity was estimated at 8.4 METhr/day (IQR: 5.1–13.7), with most of the activities performed during leisure time, followed by household activities and sport activities (Table 1).

Table 1. Differences between groups based on cutoff value of 1.0 g/kg/d

	Total population	**LPI <1.0 g/kg/d**	**HPI ≥1.0 g/kg/d**	***P*-value**
	n=140	*n*=60	*n*=80	
Age, y	83 (77–84)	83 (77–84)	83 (77–84)	0.98[2]
Male, n (%)	90 (64)	38 (63)	52 (65)	0.84[1]
Current smokers, n (%)	2 (1.4)	1 (2)	1 (1)	1.00[3]
Level of education				
Low, n (%)	14 (10.5)	6 (10)	8 (11)	
Intermediate, n (%)	78 (58.6)	36 (61)	42 (57)	0.88[1]
High / academic, n (%)	41 (30.8)	17 (29)	24 (32)	
Body composition				
Weight, kg				
Male	76.9 ± 9.4	79.5 ± 9.2	75.0 ± 9.3	**0.025**
Female	69.0 ± 12.6	74.8 ± 14.0	64.5 ± 9.4	**0.003**
BMI, kg/m^2				
Male	25.9 ± 2.7	26.4 ± 2.8	25.5 ± 2.6	0.12
Female	26.4 ± 4.7	29.0 ± 5.1	24.4 ± 3.3	**<0.001**
Dietary intake				
Energy, kcal				
Male	2040 ± 370	1842 ± 336	2185 ± 325	**<0.001**
Female	1754 ± 396	1558 ± 344	1908 ± 369	**0.001**
Carbohydrate intake, en%	43.0 ± 6.0	43.0 ± 5.8	42.9 ± 6.2	0.91
Fat intake, en%	34.5 ± 5.6	34.8 ± 6.0	34.2 ± 5.4	0.61
Total protein intake, en%	16.4 ± 3.0	15.1 ± 2.3	17.4 ± 3.1	**<0.001**
Total protein intake, g	78.9 ± 18.9	64.7 ± 12.6	89.5 ± 15.6	**<0.001**
Total protein intake, g/kg/d	1.08 ± 0.29	0.84 ± 0.13	1.27 ± 0.23	**<0.001**
Animal-based protein, %	61.8 ± 9.2	59.5 ± 8.6	63.6 ± 9.3	**0.009**
Vitamin D supplementation, n (%)	30 (21)	13 (22)	17 (21)	0.73[1]
Goldberg-score				
EI/BMR	1.34 ± 0.28	1.17 ± 0.21	1.47 ± 0.25	**<0.001**
Underreporting, n (%)	5 (4)	5 (8)	0 (0)	
Within confidence limits, n (%)	135 (96)	55 (92)	80 (100)	**0.013**[3]
Overreporting, n (%)	0 (0)	0 (0)	0 (0)	
Physical activity				
Total activity, METhr/day	8.4 (5.1–13.7)	8.4 (5.6–12.6)	8.5 (5.0–14.5)	0.93[2]
Sports, METhr/day	0.4 (0.0–1.6)	0.4 (0.0–1.6)	0.4 (0.0–1.6)	0.93[2]
Household activities, METhr/day	2.5 (0.7–5.0)	2.4 (0.4–6.2)	2.6 (1.3–4.5)	0.69[2]
Leisure time, METhr/day	3.9 (1.8–7.3)	4.2 (1.7–7.3)	3.5 (1.8–7.4)	0.85[2]

Muscle parameters				
Grip strength, kg	32 ± 10	33 ± 10	32 ± 10	0.42
SPPB total score, pt	10 (9–11)	10 (9–11)	10.5 (9–11.3)	0.15[2]
SPPB balance score, pt	4 (3–4)	4 (3–4)	4 (3–4)	0.60[2]
SPPB gait speed, s	4.0 ± 1.0	4.0 ± 1.0	4.0 ± 1.0	0.76
SPPB chair rise ability time, s	13.4 ± 4.5	13.5 ± 3.8	13.4 ± 5.0	0.85
Quality of Life				
QALY	0.96 (0.86–1.00)	0.92 (0.86–1.00)	1.0 (0.86–1.00)	0.77[2]
Health score	90 (80–95)	85 (80–94)	90 (80–95)	0.21[2]

BMI, body mass index; EI/BMR, ratio of energy intake and basal metabolic rate; en%, energy percentage; g/kg/d, gram per kilogram of body weight per day; HPI, Higher total protein intake group; LPI, Lower total protein intake group; MET, metabolic equivalent of task; N, Newton; SPPB, Short Physical Performance Battery; QALY, Quality–adjusted life year. Parametric values are means ± SDs and non-parametric values are median(IQR).

P values for differences between the two groups of total protein intake were derived by independent samples t-test unless otherwise indicated. [1] Derived by Chi-square test, [2] Derived by Kruskal-Wallis test. [3] Derived by Fisher's exact test.

Total protein intake

A total of 80 participants consumed more protein in total than 1.0 g/kg/d, whereas 60 participants consumed less than 1.0 g/kg/d (Table 1). Participants with a higher total protein intake (HPI, >1.0 g/kg/d) did not significantly differ from participants with a lower total protein intake (LPI, <1.0 g/kg/d) with respect to age, sex, smoking behaviour, level of education, vitamin D supplement use, physical activity, grip strength, SPPB scores or quality of life. In the regression analysis total protein intake was not related to SPPB total score, gait speed, chair rise ability, handgrip strength or QALY (**Table 2**).

Protein intake distribution

Figure 1 presents the distribution of protein intake of each main meal across a day for the distribution-tertiles. Average protein intake of the spread group (CV<0.43) varied less than 6.8 grams between breakfast, lunch and dinner, whereas this range was 20.9 grams and 29.3 grams for the intermediate (CV 0.43–0.62) and pulse-feeding (CV>0.62) group, respectively. The groups did not differ in age, sex, smoking behaviour, level of education, body composition, dietary energy intake, carbohydrate intake, fat intake, vitamin D supplementation, grip strength (**Figure 2**), SPPB total score

Table 2. Adjusted linear regression model* for SPPB total score, SPPB gait speed (s), SPPB chair rise (s), handgrip strength (kg) and QALY

	SPPB total score	Gait speed	Chair rise	Handgrip strength	QALY‡
Total protein intake (g/kg/d)	0.28 (-0.89 – 1.45)	0.23 (-0.41 – 0.87)	-1.78 (-5.10 – 1.43)	0.76 (-4.48 – 6.00)	-7.15 (-17.59 – 3.29)
Protein distribution					
Spread (CV<0.43)	0.62 (-0.09 – 1.33)	**-0.42 (-0.80 – -0.03)**	-1.46 (-3.41 – 0.49)	2.36 (-0.80 – 5.52)	-0.11 (-4.15– 3.94)
Intermediate (CV 0.43-0.62) *(ref)*	1.00	1.00	1.00	1.00	1.00
Pulse (CV>0.62)	0.17 (-0.54 – 0.87)	-0.18 (-0.56 – 0.21)	-0.99 (-2.93 – 0.94)	1.73 (-1.40 – 4.86)	0.07 (-3.92– 4.06)
Physical activity (METhr/day)	**0.06 (0.02 – 0.10)**	**-0.02 (-0.05 – -0.00)**	-0.07 (-0.19 – 0.05)	-0.02 (-0.21 – 0.18)	0.48 (-1.27 – 0.30)
Physical activity * total protein intake	n.s. §	n.s. §	n.s. §	n.s. §	**0.71 (0.00 – 1.41)**
Animal-based protein (%)	0.02 (-0.01 – 0.06)	-0.01 (-0.03 – 0.01)	0.03 (-0.07 – 0.12)	0.07 (-0.09 – 0.22)	0.00 (-0.20 – 0.19)

BMI, body mass index; CV, coefficient of variation; MET, metabolic equivalent of task; SPPB, Short Physical Performance Battery; QALY, Quality-adjusted life year.

*Adjusted for age, sex and BMI total protein intake, protein distribution, physical activity, interaction term between physical activity and total protein intake, animal-based protein.

§ Interaction term between physical activity and total protein intake was not significant and therefore not included in the final adjusted model.

‡ QALY was multiplied by 100.

Bold values indicate β with p-value < 0.05.

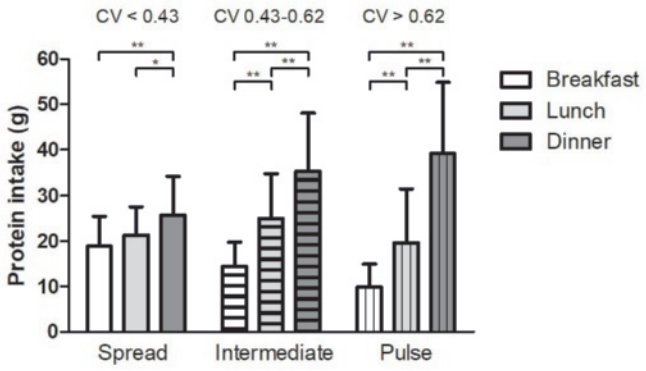


Figure 1. Protein intake during main meals of the participants in tertiles based on CV (coefficient of variance).

Participants in the spread group (n = 46, CV < 0.43) had a significantly higher protein intake at dinner compared to the protein intake at breakfast and lunch. Participants in the intermediate group (n = 48, CV 0.43–0.62) and participants in the pulse group (n = 46, CV > 0.62) had significant different intakes at all main meals. * p < 0.001, ** p = 0.011. Data are presented as means ± SDs.

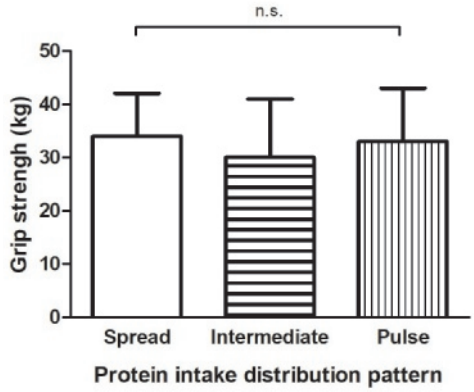


Figure 2. Hand grip strength of 3 groups based on distribution pattern of protein intake during the main meals determined with CV (coefficient of variance).

Participants in the spread group (n=46, CV <0.43), intermediate group (n=48, CV 0.43–0.62) and pulse group (n=46, CV >0.62) had similar grip strength. Data are presented as means ± SDs.

(**Figure 3A**),balance score (**Figure 3B**), chair rise ability time (**Figure 3D**), total physical activity, leisure time activity, household activity and quality of life (**Figure 4A and 4B**) (Supplemental table 1). Sports activity was significantly higher in the spread- and intermediate groups compared to the pulse group (Δ0.7 METhr/day, *P*=0.022 and Δ0.6 METhr/day, *P*=0.044 respectively, **Supplemental table 1**) and gait speed was significantly higher in the spread distribution group compared to the intermediate group (Δ0.5 s, *P*=0.040, **Figure 3C** and Supplemental table 1). This was confirmed in the adjusted regression model in which a more spread protein distribution was related to a higher gait speed as opposed to the intermediate distribution group (β=–0.42, *P*=0.035, **Table 3**).

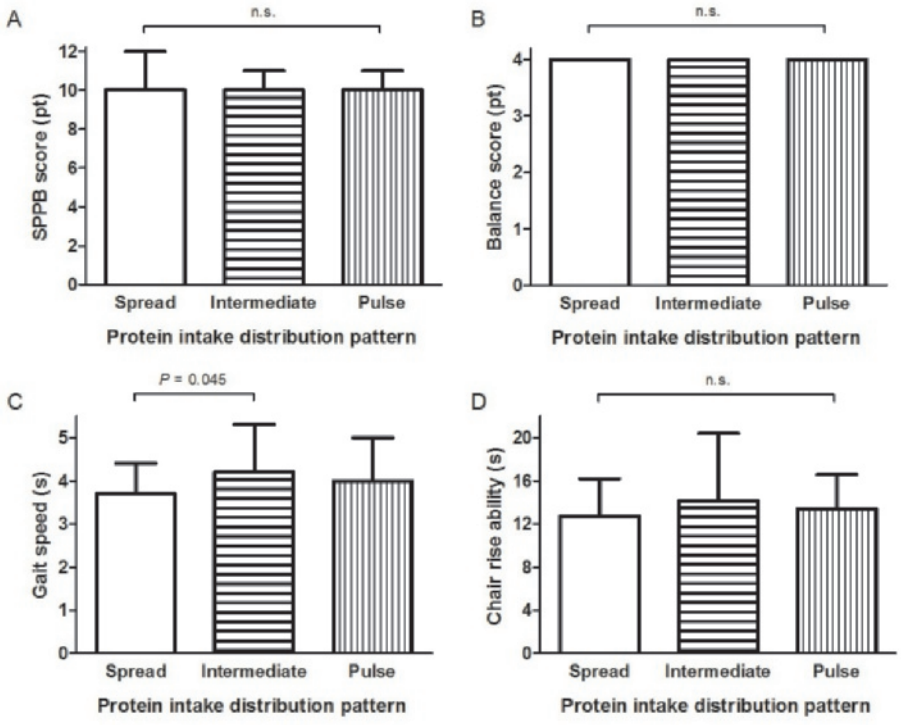


Figure 3. SPPB total score (A), balance score (B), gait speed (C) and chair rise ability (D) of 3 groups based on distribution pattern of protein intake during the main meals determined with CV (coefficient of variance).

Participants in the spread group (*n*=46, CV <0.43), intermediate group (*n*=48, CV 0.43–0.62) and pulse group (*n*=46, CV >0.62) had similar scores for SPPB (A) and balance (B) and similar chair rise ability (D). Gait speed was significantly higher in the spread distribution group (3.7 ± 0.7) compared to the intermediate group (4.2 ± 1.1) *P*=0.045. Data are presented as means ± SDs.

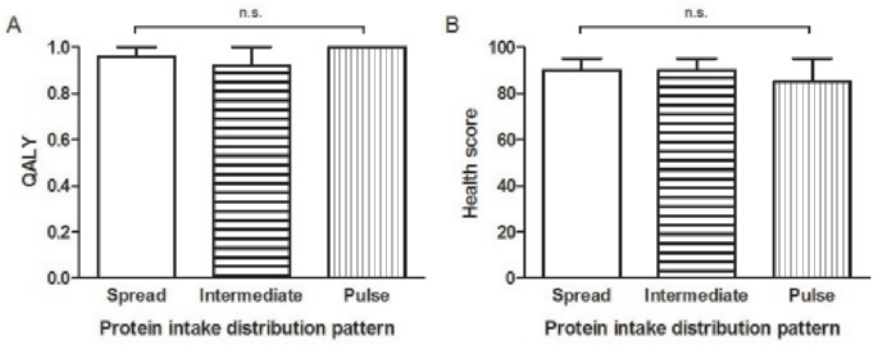


Figure 4. QALY (A) and health score (B) 3 groups based on distribution pattern of protein intake during the main meals determined with CV (coefficient of variance).

Participants in the spread group (*n*=46, CV <0.43), intermediate group (*n*=48, CV 0.43–0.62) and pulse group (*n*=46, CV >0.62) had similar QALY and health scores. Data are presented as means ± SDs.

Effect of concurrent physical activity and total protein intake

The interaction between physical activity and total protein intake was positively associated with QALY (β=0.71, *P*=0.049), whereas physical activity or total protein intake individually were not significantly related to QALY. No significant relation was found for the interaction between physical activity and total protein intake with grip strength, SPPB total score, balance score, chair rise ability time, gait speed (Table 2).

DISCUSSION

In contrast to our hypotheses the results of our study show that in a sample of community-dwelling elderly with a wide range of physical activity, total protein intake was not associated with muscle strength, physical function or quality of life. Nevertheless, a spread distribution of protein intake during the main meals as opposed to an intermediate feeding pattern was related to a higher gait speed. The interaction between physical activity and total protein intake was related to higher quality of life.

Total protein intake

In our study we observed no association of total protein intake with SPPB scores, handgrip strength and quality of life. The absence of a positive effect of a higher total protein intake on these parameters might be explained by the fact that the contrast in total protein intake between the two groups was rather small, and the average daily dietary intake of 1.1 ± 0.3 g protein/kg/d was well above the Recommended Dietary Allowance (RDA) of 0.8 g/kg/d. In

our group analysis comparing lower *versus* higher total protein intake, we used the cut-off value of 1.0 g/kg/d, a value that has been suggested to be the RDA for total protein intake in elderly [6, 35, 36]. Studies with larger sample sizes have indeed shown that intakes above 1.0 g/kg/d attenuate the decline in muscle mass and function in elderly [37, 38]. Moreover, Granic *et al*. [39] showed that an intake below 1.0 g/kg/d can negatively affect grip strength or physical functioning. On the contrary, a recent meta-analysis found no effect of protein or amino acid supplementation on muscle mass or strength in mostly non-frail elderly with an average habitual total protein intake of 1.0 g/kg/d [40]. In our non-frail elderly population with on average a total protein intake well above the current RDA for protein, no additional beneficial effects for muscle strength or physical functioning were observed in those with a higher total protein intake.

Protein intake distribution

We determined tertiles of distribution based on a continuous measure (CV) by which we avoided the use of arbitrary cutoff values. In our adjusted regression model a spread protein intake pattern over the main meals was positively associated with gait speed as opposed to an intermediate pattern of intake. Gait speed in an elderly population is a strong predictor of survival [41] and is therefore an important marker of overall health. The proposed beneficial effects of a spread protein intake pattern over the main meals are in line with several studies in frail elderly that have demonstrated benefits of a more evenly distributed protein intake on frailty, muscle protein synthesis, and lean body mass [14-17]. Recommendations for a spread protein intake state that mealtime intake should be at least 25-30 grams [42-47]. Our group with a spread protein intake had an average intake of 19 gram, 21 gram and 26 gram for breakfast, lunch and dinner, respectively (Figure 1 & Supplemental table 1). The benefit of a spread distributed protein intake may even be higher with mealtime intakes reaching the 25-30 gram threshold.

We found no positive effects of a pulse-feeding pattern compared to the intermediate cluster, whereas previous studies reported benefits of pulse feeding [12,13]. The fact that our CV for pulse-feeding pattern was lower than the CV defined by Cardon-Thomas *et al*. [48] and that the protein intake in our study was lower and less concentrated in one meal than presented in previous studies [12,13], suggests that our pulse feeding group did not completely comply to pulse feeding strategies used in other studies, and therefore we should be cautious with the findings in our study that pulse feeding had no effect on any of the outcomes.

Concurrent effect of physical activity and total protein intake

The combination of physical activity and total protein intake was positively associated with overall quality of life in our adjusted model. Most studies that assessed the association between nutrition and quality of life, focus on nutritional status or malnutrition in frail or

hospitalized populations [49-51]. While these studies do find a positive association between total protein intake and quality of life, no relationship was present in our study with total protein intake only. However the combination of higher physical activity and higher total protein intake was positively associated with improved quality of life in physically active elderly people. A randomized controlled trial also found an increase in quality of life after participants performed resistance exercise training while increasing their protein intake [52]. These results emphasize the need of combining sufficient high total protein intake with an active lifestyle in elderly.

Strengths and limitations

The strengths of this study were that we have a sample of community-dwelling elderly with a high mean age (80+) and a broad range of physical activity levels (0 – 35 METhr/wk). Furthermore, we use a relevant set of validated outcomes, including objective measures (physical function, strength) and self-reported quality of life. Assessing physical activity with a questionnaire elicits less reliable measurements when compared to using activity monitors, but these validated questionnaires provide a representative estimate of differences in physical activity between participants [31,32]. The cross-sectional design of the study limits the ability to assess causality. Furthermore, we have used data from two different studies that used different methods to assess dietary intake and physical activity. However, the dietary intake measures are comparable, and we coded and calculated the dietary intake and physical activity data in the same way, which allowed us to combine the two studies and present a unique population of elderly people with a broad range of physical activity.

Conclusion

A higher total protein intake was not associated with improved physical outcome measures. A more spread protein intake during the main meals was related to a higher gait speed, an important measure of survival in the elderly. In addition, combining higher physical activity with higher total protein intake is related to a better quality of life, emphasizing the need for a higher total protein intake together with an active lifestyle in the elderly.

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SUPPLEMENTAL MATERIAL

Supplemental table 1. Tertiles based on distribution pattern of protein intake (CV).

	Spread (<0.43) *n*=46	**Intermediate** (0.43-0.62) *n*=48	**Pulse** (>0.62) *n*=46	**P-value**
Age, y	83 (81-86)	82(75-83)	83(78-84)	0.10[1]
Male, %	34 (74)	27 (56)	29 (63)	0.20[1]
Current smokers, n (%)	1 (2)	1 (2)	0 (0)	0.68[3]
Level of education				0.86[1]
Low, n (%)	4 (9)	6 (13)	4 (9)	
Intermediate, n (%)	27 (61)	24 (52)	27 (63)	
High / academic, n (%)	13 (30)	16 (35)	12 (28)	
Body composition				
Weight, kg				
Male	76.3 ± 9.0	76.2 ± 11.3	78.3 ± 8.2	0.64
Female	65.9 ± 7.6	67.9 ± 12.7	72.9 ± 14.9	0.28
BMI, kg/m2				
Male	25.6 ± 2.6	25.9 ± 3.0	26.3 ± 2.6	0.61
Female	25.0 ± 2.9	26.3 ± 5.0	27.6 ± 5.3	0.34
Dietary intake				
Energy, kcal				
Male	2032 ± 388	2115 ± 374	1981 ± 342	0.38
Female	1818 ± 411	1783 ± 405	1673 ± 383	0.58
Carbohydrate intake, en%	42.5 ± 6.2	43.6 ± 5.9	42.7 ± 5.9	0.34
Fat intake, en%	35.4 ± 6.5	34.3 ± 4.7	33.7 ± 5.5	0.66
Protein intake, en%	15.6 ± 2.4	17.0 ± 3.7	16.7 ± 2.6	0.06
Protein intake, g	76.8 ± 18.4	82.2 ± 19.7	77.5 ± 18.5	0.28
Protein intake at breakfast, g	18.9 ± 6.5	14.4 ± 5.4	9.8 ± 5.1	**<0.001**
Protein intake at lunch, g	21.4 ± 6.2	25.0 ± 9.7	19.6 ± 11.9	**0.023**
Protein intake at dinner, g	26.1 ± 8.5	35.3 ± 12.8	39.2 ± 15.7	**<0.001**
Protein intake, g/kg/d	1.05 ± 0.27	1.16 ± 0.31	1.04 ± 0.28	0.09
Animal-based protein, %	58.6 ± 8.5*	62.5 ± 9.4	64.4 ± 9.0*	**0.009**
Vitamin D supplementation, n (%)	9 (20)	11 (23)	10 (22)	0.89[1]
Goldberg-score				
EI/BMR	1.35 ± 0.26	1.40 ± 0.28	1.28 ± 0.28	0.11
Underreporting, n (%)	0 (0)	2 (4)	3 (7)	
Within confidence limits, n (%)	46 (100)	46 (96)	43 (93)	0.29[3]
Overreporting, n (%)	0 (0)	0 (0)	0 (0)	

Physical activity				
Total activity, METhr/day	8.2 (5.8-12.6)	9.0 (5.0-15.4)	8.4 (4.7-12.2)	0.89[2]
Sports, METhr/day	0.7 (0.0-1.7)	0.6 (0.0-1.9)	0.0 (0.0-0.7)	**0.045[2]**
Household activities, METhr/day	1.8 (0.3-3.6)	3.2 (1.3-6.1)	3.5 (0.6-5.7)	0.13[2]
Leisure time, METhr/day	4.8 (2.5-8.2)	3.4 (1.3-7.1)	3.0 (2.0-6.7)	0.28[2]
Muscle parameters				
Grip strength, N	34 ± 8	30 ± 11	33 ± 10	0.17
SPPB total score	11 (10-12)	10 (9-11)	10 (9-11)	0.22[1]
SPPB balance score	4 (3-4)	4 (3-4)	4 (3-4)	0.90[1]
SPPB gait speed, s	3.7 ± 0.7*	4.2 ± 1.1*	4.0 ± 1.0	**0.045**
SPPB chair rise ability time, s	12.7 ± 3.5	14.2 ± 6.2	13.4 ± 3.2	0.27
Quality of Life				
QALY	0.92 (0.88-1.0)	1.0 (0.86-1.0)	0.92 (0.86-1.0)	0.86
Health score	90 (80-95)	90 (80-95)	85 (75-95)	0.25

BMI, body mass index; CV, coefficient of variation; EI/BMR, ratio of energy intake and basal metabolic rate; en%, energy percentage; g/kg/d, gram per kilogram of body weight per day; MET, metabolic equivalent of task; N, Newton; SPPB, Short Physical Performance Battery; QALY, Quality-adjusted life year. Parametric values are means ± SDs and non-parametric values are median(IQR).

P values for differences between the two groups of protein intake were derived by independent samples t-test unless otherwise indicated. [1] Derived by Chi-square test, [2] Derived by Kruskal-Wallis test. [3] Derived by Fisher's exact test.* Significant difference between tertiles.

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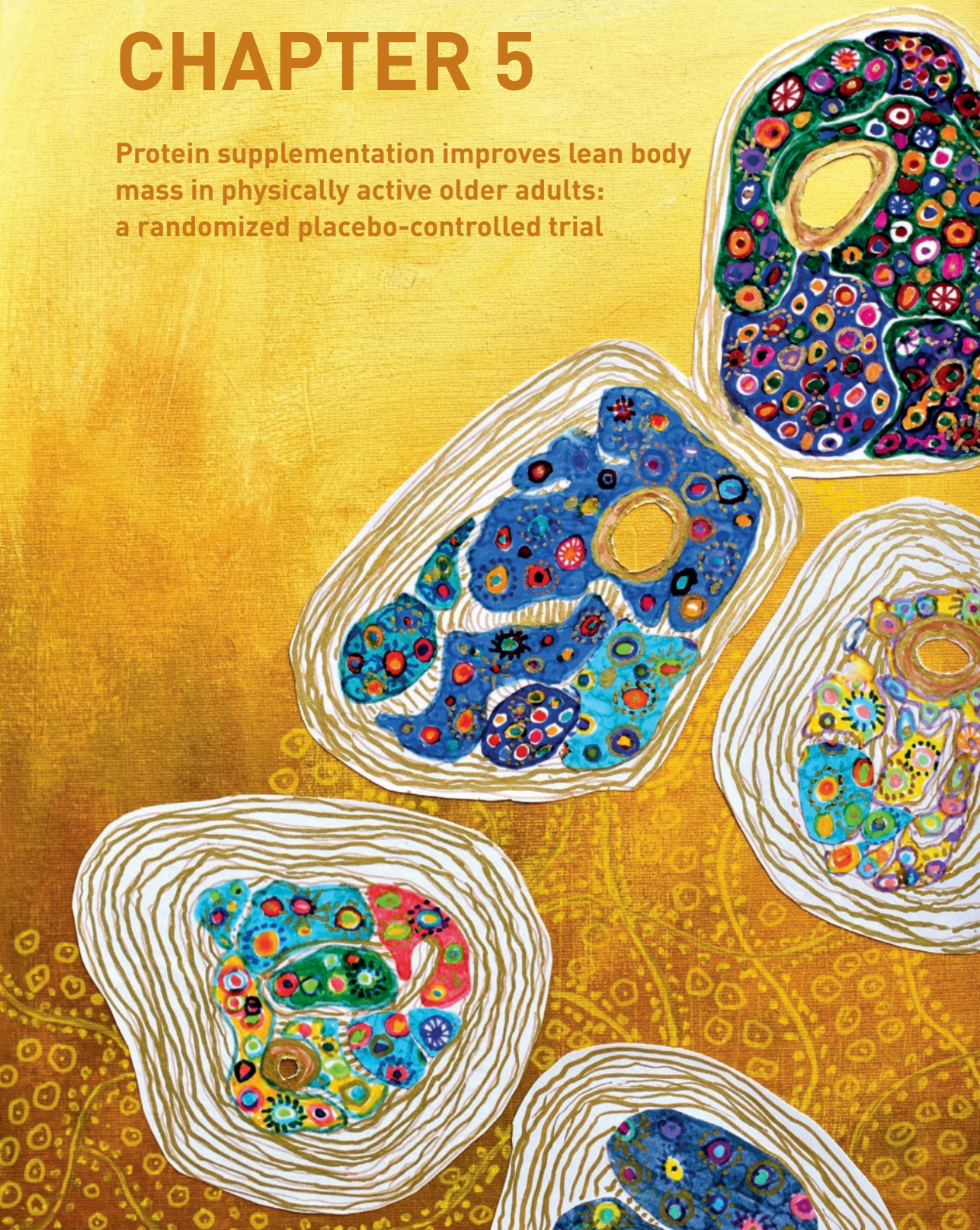
CHAPTER 5

Protein supplementation improves lean body mass in physically active older adults: a randomized placebo-controlled trial



ABSTRACT

Background: An inadequate protein intake may offset the muscle protein synthetic response after physical activity, reducing the possible benefits of an active lifestyle for muscle mass. We examined the effects of 12 weeks of daily protein supplementation on lean body mass, muscle strength and physical performance in physically active older adults with a low habitual protein intake (<1.0 g/kg/day).

Methods: A randomized double-blinded controlled trial was performed among 116 physically active older adults (age 69 (Interquartile range: 67 – 73) y, 82% male) who were training for a 4 day walking event of 30, 40 or 50 km/day. Participants were randomly allocated to either 31 g of milk protein or iso-caloric placebo supplementation for 12 weeks. Body composition (dual-energy X-ray absorptiometry), strength (isometric leg extension and grip strength), quadriceps contractile function, and physical performance [Short Physical Performance Battery, Timed Up-and-Go test and cardiorespiratory fitness (Åstrand-Rhyming submaximal exercise test)] were measured at baseline and after 12 weeks. We assessed vitamin D status and markers of muscle damage and renal function in blood and urine samples before and after intervention.

Results: A larger increase in relative lean body mass was observed in the protein vs. placebo group (Δ0.93 ± 1.22% vs.Δ0.44 ± 1.40%, $P_{Interaction}$ = 0.046). Absolute and relative fat mass decreased more in the protein group than in the placebo group (Δ-0.90 ± 1.22 kg vs.Δ-0.31 ± 1.28 kg, $P_{Interaction}$ = 0.013 and Δ-0.92 ± 1.19% vs.Δ-0.39 ± 1.36%, $P_{Interaction}$ = 0.029, respectively). Strength and contractile function did not change in both groups. Gait speed, chair-rise ability, Timed Up-and-Go, and cardiorespiratory fitness improved in both groups (P < 0.001), but no between-group differences were observed. Serum urea increased in the protein group whereas no changes were observed in the placebo group ($P_{interaction}$ < 0.001). No between-group differences were observed for vitamin D status, muscle damage and renal function markers.

Conclusions: In physically active older adults with relatively low habitual dietary protein consumption, an improvement in physical performance, an increase in lean body mass, and a decrease in fat mass were observed after walking exercise training. A larger increase in relative lean body mass and larger reduction in fat mass were observed in participants receiving 12 weeks of daily protein supplementation compared with controls, whereas this was not accompanied by differences in improvements between groups in muscle strength and physical performance.

INTRODUCTION

A physically active lifestyle attenuates the age-related loss of muscle mass (*i.e.* sarcopenia) and associated decrements of muscle function (1, 2) by increased muscle protein synthesis rates after exercise but also due to preservation of skeletal muscle sensitivity to dietary amino acids and suppressing the catabolic inflammatory cytokines in the muscle (3-5). Sufficient protein intake is another vital component to maintain and regain muscle mass (6-8). Current recommendations for adults advice 0.8 g/kg/d (9). However, the PROT-AGE study group suggested that older adults above 65 years of age should consume 1.0-1.2 g/kg/d to compensate for the attenuated capacity of protein utilization in the aging muscles (6). For physically active older adults their recommendation is even higher, *i.e.*, ≥1.2 g/kg/d in order to comply with the synergistic effects of exercise and protein intake on muscle protein synthesis (6). It has previously been shown that more than 50% of physically active older adults has a protein intake below 1.2 g/kg/d (10). This observation suggests that physically active older adults may not consume enough protein to be utilized for the exercise-induced improved muscle protein synthetic response and, thus, to prevent age-related muscle mass loss.

Therefore, we assessed the effects of 12 weeks of daily protein supplementation on lean body mass, muscle strength, and physical performance in physically active older adults with a low habitual protein intake. We hypothesized that protein supplementation in physically active older adults would induce beneficial effects on lean body mass, muscle strength and physical performance while no effects were expected in the control group receiving an iso-caloric placebo.

METHODS

Participants

Participants were recruited between March 16, 2017 and April 12, 2017 via the Nijmegen Exercise Study database [Study-ID: NL36743.091.11 (11)] and social media. Interested men and women of at least 65 years were included if they i) had a habitual protein intake ≤ 1.0 g/kg/d based on a 123 item online food frequency questionnaire (FFQ) (12) calculated using the Dutch Food composition database of 2010 (13), ii) were registered and training for the 2017 Nijmegen Four Days Marches [an annual 4 day walking event (30, 40 or 50 km/day) in the Netherlands; https://www.4daagse.nl/en], and iii) were able to understand and perform the study procedures. Exclusion criteria for participation in the study were type 1 or type 2 diabetes mellitus (non-fasted state >11 mmol/L), allergic or sensitive for milk proteins or lactose intolerant, Chronic Obstructive Pulmonary Disease, cancer, renal insufficiency [estimated Glomerular filtration rate (eGFR) <30 ml/min/1.73m^{-1}], intestinal diseases that may influence the uptake of protein,

use of statins, and involved in a heavy resistance type exercise program. All participants signed an informed consent form prior to any experimental procedure. The study conformed to the principles of the Declaration of Helsinki and was approved by a Medical Ethical committee, the Independent Review Board Nijmegen (Study-ID: NL60137.072.16). This trial was registered at www.trialregister.nl as NTR6488.

Design

In a double-blind, controlled intervention study a total of 116 eligible participants were randomly allocated to either the protein-supplemented or the placebo-supplemented group. An independent researcher randomized the study participants by means of computer-generated random numbers with a block size of 10 in a 1:1 ratio. Before and after 12 weeks of supplementation, anthropometrics, dual-energy X-ray absorptiometry (DXA), strength measurements (maximal isometric leg extension and handgrip strength) and physical performance measurements [Short Physical Performance Battery (SPPB), Timed Up-and-Go (TUG) and the Åstrand-Rhyming submaximal exercise test] were performed. Additional muscle function measurements were performed in a subgroup of 30 participants of the protein group and 30 participants of the placebo group. Blood samples, dietary intake (24 hr recall) and physical activity (Short Questionnaire to Assess Health enhancing physical activity (SQUASH)) data were collected from all participants. In addition, participants were invited to complete an online diary every week, reporting their daily supplement intake and training kilometers for the Nijmegen Four Days Marches.

Protein intervention

Participants were asked to consume either a 250 mL protein supplement or a 250 mL iso-caloric placebo drink, twice a day. Two packages of the protein supplement (500 mL) contained in total 36.8 g milk protein concentrate (MPC 80) with 31 g protein, 1.1 g fat and 14.5 g lactose (carbohydrates), whereas 500 mL of the placebo supplement contained 1.1 g protein, 5.2 g fat and 36 g of carbohydrates (FrieslandCampina Consumer Products Europe, Wageningen, the Netherlands). Protein and placebo supplements were provided in ready-to-drink non-transparent packages of 250 mL and were vanilla flavoured to mask contents. Participants were asked to consume one beverage during breakfast and one beverage within 30 minutes after exercise (e.g. walking). On non-exercising days, participants were instructed to consume the second beverage during lunch. Participants were asked to report their daily supplement intake every week. Compliance was calculated by dividing the number of used supplements by the total supplements and multiplied by 100. Adverse events were documented.

Measurements

Body composition. Height and weight (Seca 888 scale, Hamburg, Germany) were measured and used to calculate the body mass index (BMI). Total and regional lean body mass and fat mass of the participants were measured by DXA (Lunar Prodigy Advance DXA; GE Healthcare, Madison, WI, USA). The DXA scans were performed with dual energy beam (0.03 mrem) and a scan time of approximately 10 minutes.

Handgrip strength. Handgrip strength of the dominant hand was measured with a hydraulic, analogue hand held dynamometer (Jamar®, Jackson, MI, USA). For every participant the dynamometer was adjusted to their hand size. The participants were seated in a chair without arm rests with the elbow flexed in a 90° angle position and were asked to shortly maximally squeeze the handgrip instrument three times with 1 minute rest between measurements. The maximum strength in kilograms was used for analysis.

Quadriceps strength and contractile function. Additional validated muscle characteristic measurements (14) were performed in a subgroup of 30 participants of the protein group and 30 participants of the placebo group. Muscle strength was measured by performing three to six isometric maximal voluntary contractions (MVC) of the dominant quadriceps femoris muscle for approximately 3 s (15). The force signal was amplified (strain indicator type CA660, Peekel Instruments, Rotterdam, the Netherlands), digitized (1000Hz) and stored. The highest MVC was expressed absolute and relative to body weight. Electrically stimulated quadriceps muscle contractions were obtained at 40% of the MVC with 1 s 50 Hz electrical impulses generated by a direct-current high-voltage stimulator (DS7A, Digitimer Ltd, Hertfordshire, UK), through two surface electrodes on the distal and proximal part of the anterior thigh (Electro-Medical Supplies, Greenham Ltd, Wantage, Oxfordshire, UK) to assess voluntary muscle strength, function and fatigue (15). A force-frequency relationship of only the valid measurements that were not limited by technical constraints were obtained through peak force generation upon five 1 s stimulation frequencies (1, 10, 30, 50 and 100 Hz, respectively). Contraction and relaxation rates were calculated as indices of muscle speed of the average of 1, 30, 50 and 100 Hz impulse; normalized maximal rate of force rise was expressed as the maximal slope of force increment as percentage of peak force (16), and early- and half-relaxation time was defined as the time taken for force to decline from 75% to 50% and from 50% to 25% of the peak force, respectively. Resistance to fatigue was assessed by activating the quadriceps muscle repetitively using 30 Hz bursts with a 1 s duration every 2 s for 2 min. Only the valid muscle fatigue resistance measurements were expressed as a percentage of average force of the last three contractions from the average force of the first 3 contractions and the peak force per repetition was analysed.

Short Physical Performance Battery (SPPB). Physical performance was assessed using the SPPB, which is considered a reliable and valid method in older adults (17). The SPPB consists of three components for which 0-4 points could be earned: balance, gait speed and chair rise ability. Participants' balance was assessed by examining their ability to stand still for 10 s with

their feet side by side, in semi-tandem and in tandem position. Gait speed was determined by the time necessary to complete a walk of 4 m on their usual gait speed. The chair rise ability score was determined by the time necessary to rise out of a chair and sit down five times in a row, without aid of arms. For gait speed and chair rise ability the quickest time out of two attempts was reported. A SPPB total score (0-12 points) was calculated by summing the scores.

Timed Up-and-Go (TUG) test. During the TUG test the participants were instructed to rise from a chair, walk 3 m, turn around, walk back and sit down again as quickly as possible (18, 19). The time was reported after one trial run.

Åstrand-Rhyming test. To evaluate cardiorespiratory fitness, participants performed the Åstrand-Rhyming submaximal exercise test on a stationary bicycle. The test was performed on a mechanically braked cycle ergometer (Corival model, Lode Holding Company BV, the Netherlands) and heart rate was measured with a Polar (Polar Electro, RS400 and RS800 model, Kempele, Finland). The maximal volume of oxygen consumption (VO_2max) was estimated by applying the work rate and mean heart rate of the 5th and 6th minute to the Åstrand normogram, with correction for weight and age (20, 21).

Physical activity. Habitual physical activity was assessed at baseline by the SQUASH questionnaire, which is considered a valid and reliable method in older adults (22). This self-administered questionnaire estimates habitual level of physical activity during a normal week over the past month, with questions about the type, duration and frequency of activities. Total physical activity and exercise-specific activities were calculated in metabolic equivalent of task hours per day by multiplying the exercise time in hours with the accompanying metabolic equivalent of task score of the activity (23). Moreover, participants reported their weekly walking exercise (in kilometres) as a training for the Nijmegen Four Days Marches.

Dietary intake. Daily dietary intake was assessed using a repeated 24 h recall, which is a validated method to assess the amount and distribution of protein intake (24). Two recall days were randomized over the week with the restriction that no participant was assigned to two identical week days or two weekend days. The 24 h recall was performed face-to-face or by phone by trained dieticians and coded by the same dieticians into the web-based program Compl-eat, which calculated the dietary intake using the Dutch Food Composition Database of 2016 (25). The mean of the two recorded days represented the daily dietary intake.

Blood samples. Non-fasted venous blood was drawn from the antecubital vein before and after the supplementation period, and serum and lithium heparin samples were stored at -80 ^{o}C until analysis. Non-fasting glucose and creatinine levels were assessed to calculate eGFR and were analyzed at baseline to exclude participants suffering from insulin resistance, type II diabetes and renal insufficiency. To check protein intake and renal function before and after the supplementation period, we assessed urea, creatinine, and albumin concentrations. Moreover we assessed creatine kinase to identify if muscle damage occurred (26). Vitamin D status, C-reactive protein (CRP), Interleukin (IL)6 and IL10 were assessed, because of their possible

confounding effects on muscle mass (27, 28). Glucose, creatinine, urea, albumin, creatine kinase, and CRP were measured using Siemens Dimension Vista 1500 (Siemens Healthcare Diagnostics Inc., Tarrytown, New York, USA). Serum 25-hydroxyvitamin D concentrations were measured using liquid chromatography coupled to tandem mass spectrometry detection (Waters Chromatography B.V., Etten-Leur, the Netherlands). Serum IL-6 and IL-10 concentrations were determined using a multiplex electroluminescence-based cytokine assay on a MESO QuickPlex SQ120 plate imager (Meso Scale Diagnostics, Rockville, Maryland, USA). Analysis were performed by trained technicians using standard operating procedures, on a single day using the same calibration and set-up to minimize variation.

Urine analysis. Upon arrival in the laboratory, a urine sample (5 mL) was provided by all participants and was frozen and stored at -80°C. After completion of the study baseline and post-supplementation albumin and creatinine were determined to assess renal function using Dimension Vista 1500 (Siemens Healthcare Diagnostics Inc.).

Statistical analysis

Based on a Type I-error of 0.025 and a power of 90% we calculated (G-power, version 3.1.2, University of Dusseldorf, Germany) that 53 participants per study arm were needed to find an expected difference in changes in quadriceps strength of 5 ± 5 kg and 0.41 ± 0.65 in SPPB score between the protein and placebo group (29). To account for potential drop out (~10%), we recruited 58 participants per study arm in our study. Statistical analyses were performed using SPSS 22.0 software (IBM SPSS Statistics for Windows, Version 22.0 IBM Corp., Armonk, NY, USA). A per-protocol analysis was used including only those participants with a compliance rate of ≥ 90%. All continuous variables were visually inspected and tested for normality with the Shapiro-Wilk test. Participant characteristics were displayed as mean ± SD or mean ± SE or median [interquartile range (IQR)] for parametric and non-parametric continuous variables respectively, and as number of participants with percentages for categorical variables. Baseline characteristics between groups were compared by means of an independent-samples *t*-test or a Mann-Whitney U test for parametric or non-parametric continuous variables, respectively or with a chi-square test for categorical variables. Data from before and after the supplementation period were analysed by using repeated-measures analysis of variance with time as a within-subjects factor and treatment as a between-subjects factor. Because no between-group differences were found at baseline, no variables were added as a confounder in the main analysis. The level of significance was set at $p < 0.05$ (two-sided).

RESULTS

Participants

For this study, 177 participants were screened and 116 participants were included in the study and randomly allocated to the protein or placebo group. One participant had elevated blood glucose levels and was therefore excluded from the study and another participant dropped out after 2 weeks due to gastrointestinal complaints (**Figure 1**). There were no differences between the protein and placebo group for any of the baseline characteristics (**Table 1**). Almost all participants were Caucasian, except for one Asian participant of the protein group. Six participants experienced gastrointestinal complaints during the supplementation period (3 participants of the protein and 3 participants of the placebo group), but did not drop out. There were no serious adverse events reported during the supplementation period. Compliance of supplementation intake was high and did not differ between the protein and placebo group (96 ± 3% and 95 ± 3%, respectively).

Protein intake

Protein intake was comparable between the protein and placebo group at baseline ($P = 0.18$), with more than 60% derived from animal proteins in both groups (Table 1). A significant increase in protein intake (i.e. excluding supplements) was observed over time ($P_{Time} = 0.034$) but no differences were observed between groups (**Table 2**). Daily energy and macronutrient intake did not differ between groups at baseline and did not change over time (Table 1 and 2). Taking into account the protein supplements, total protein intake increased in the protein group to 1.29 ± 0.28 g/kg/d during the 12 week supplementation period.

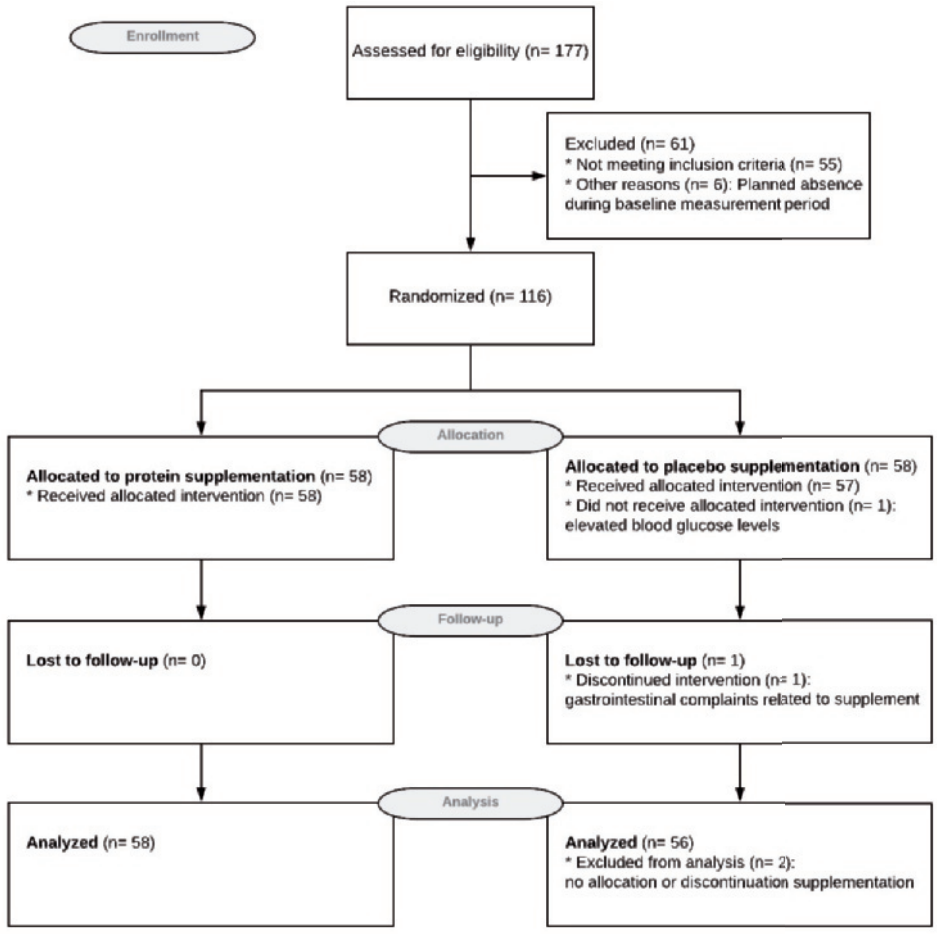


Figure 1. CONSORT flow diagram illustrating the movement of participants through the study, which was conducted between March 2017 and July 2017.

Table 1. Baseline characteristics of participants in the protein and placebo group

	Total group *n*=114	**Protein** *n*=58	**Placebo** *n*=56	**P-value**
Demographics				
Age, years	69 (67 – 73)	69 (67 – 72)	69 (67 – 73)	0.82*
Male, n (%)	93 (82)	47 (81)	46 (82)	0.88‡
Body composition				
Body weight, kg	83.1 ± 10.4	84.6 ± 10.2	81.5 ± 10.5	0.11§
BMI, kg/m^2	26.8 ± 2.6	27.2 ± 2.6	26.3 ± 2.5	0.05§
Waist-hip ratio	0.94 ± 0.08	0.95 ± 0.07	0.94 ± 0.08	0.42§
Diet				
Energy intake, kcal	1944 ± 533	1919 ± 534	1970 ± 536	0.61§
Protein intake, g/kg/d	0.89 ± 0.23	0.86 ± 0.23	0.92 ± 0.24	0.18§
Animal protein, %	61.2 ± 11.1	61.5 ± 11.4	61.1 ± 10.9	0.73§
Plant protein, %	38.8 ± 11.1	38.5 ± 11.4	39.0 ± 10.9	0.73§
Protein, en%	16.0 ± 3.4	16.2 ± 3.1	15.7 ± 3.6	0.44§
Fat intake, en%	35.6 ± 6.7	35.7 ± 7.0	35.5 ± 6.5	0.88§
Carbohydrate intake, en%	42.3 ± 7.3	42.4 ± 8.1	42.1 ± 6.4	0.81§
Physical activity				
Total physical activity, METhr/wk	117.7 (81.7 – 173.5)	109.0 (79.1 – 142.1)	124.0 (87.3 – 186.1)	0.14*
Domestic work activities, METhr/wk	26.3 (11.3 – 45.1)	22.5 (6.3 – 41.4)	29.5 (15 – 48.2)	0.14*
Commuting activities, METhr/wk[a]	0.0 (0.0 – 0.0)	0.0 (0.0 – 0.0)	0.0 (0.0 – 0.0)	0.73*
Leisure time activities, METhr/wk	53.4 (38.3 – 73.1)	50.8 (33.0 – 70.0)	59.3 (39.9 – 77.9)	0.22*
Sports activities, METhr/wk	21.0 (3.4 – 41.2)	21.0 (0.0 – 39.7)	18.2 (7.8 – 51.0)	0.74*
Blood analysis				
eGFR, ml/min/1.73m^{-1}	81.2 ± 11.6	79.4 ± 13.5	83.0 ± 9.1	0.11§
Non-fasted glucose, mmol/L[b]	5.8 ± 1.1	5.7 ± 1.1	5.8 ± 1.2	0.52§
25(OH)D, nmol/L[b]	73.7 ± 27.2	73.7 ± 28.9	73.8 ± 25.6	0.98§
CRP, mg/L	3.9 ± 3.3	4.0 ± 3.8	3.7 ± 2.9	0.66§
IL-6, pg/mL	1.02 ± 2.71	0.64 ± 0.44	1.41 ± 3.82	0.13§
IL-10, pg/mL	0.305 ± 0.438	0.327 ± 0.446	0.282 ± 0.431	0.58§

Data are presented as number (percentage) of participants, mean ± standard deviation or median (interquartile range). 25(OH)D, 25-hydroxyvitamin D; BMI, Body mass index; CRP; C-reactive protein; eGFR, estimated glomerular filtration rate; en%, energy percentage; IL, Interleukin; MET, metabolic equivalent of task. [a] *n* = 22. [b] *n* = 113

§ Derived by independent-samples t-test. * Derived by Mann-Whitney U test. ‡ Derived by Chi-square test.

Table 2. Changes in habitual dietary intake of participants in the protein and placebo group (disregarding supplements)

	Protein n=58			**Placebo** n=56			**P-value**		
	Pre	Post	Change	Pre	Post	Change	Time	Treatment	Interaction
Energy intake, kcal	1919 ± 534	1841 ± 456	-77.8 ± 484.5	1970 ± 536	1960 ± 492	-10.4 ± 535.6	0.36	0.30	0.48
Protein intake, g/kg/d	0.86 ± 0.23	0.92 ± 0.27	0.06 ± 0.27	0.92 ± 0.24	0.97 ± 0.23	0.05 ± 0.28	**0.034**	0.18	0.74
Protein intake at breakfast, g	11.3 ± 4.8	11.8 ± 7.6	0.5 ± 7.0	12.9 ± 7.7	13.4 ± 7.2	0.3 ± 8.1	0.62	0.17	0.90
Protein intake at lunch, g	21.7 ± 10.2	20.3 ± 14.5	-3.7 ± 9.3	18.3 ± 7.8	20.7 ± 9.5	2.5 ± 11.6	0.57	0.61	**0.003**
Protein intake at dinner, g	31.0 ± 10.8	33.8 ± 16.8	2.8 ± 19.5	36.0 ± 14.1	37.4 ± 13.4	1.7 ± 21.3	0.25	**0.014**	0.77
Protein, en%	16.2 ± 3.1	16.8 ± 3.8	0.6 ± 3.8	15.7 ± 3.6	16.4 ± 3.0	0.7 ± 3.9	0.07	0.41	0.88
Fat, en%	35.7 ± 7.0	35.5 ± 7.1	-0.2 ± 8.0	35.5 ± 6.5	36.6 ± 6.4	1.1 ± 7.5	0.53	0.64	0.36
Carbohydrate, en%	42.4 ± 8.1	42.6 ± 7.7	0.1 ± 8.6	42.1 ± 6.4	40.9 ± 7.4	-1.2 ± 8.0	0.51	0.40	0.40

Data are presented as mean ± standard deviation. Bold values indicate p-value < 0.05. en%, energy percentage.

Physical activity

Participants of the protein and control group reported a similar physical activity volume at baseline ($P = 0.14$, Table 1). All participants performed walking exercise training as a preparation for the Nijmegen Marches. Significant changes over time were observed in training kilometers ($P_{Time} < 0.001$, **Figure 2**), but no between-group differences were observed ($P_{Interaction} = 0.85$). The sum of walking kilometers during the 12 weeks of the study was not different between groups (protein: 391 (IQR: 286 – 512) km *versus* placebo: 338 (IQR: 239 – 493) km, $P = 0.31$).

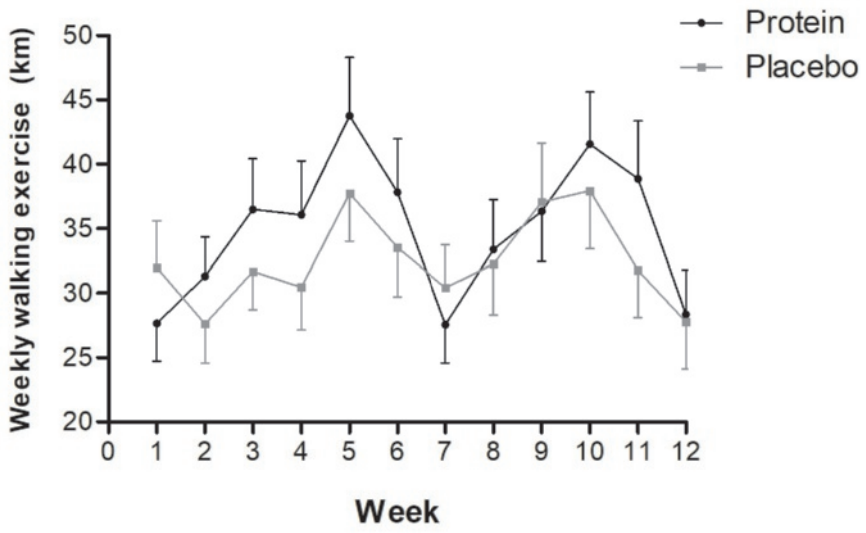


Figure 2. Training walking exercise plotted for every week in kilometres for the protein group, $n = 58$, black lines and for the placebo group, $n = 56$, grey lines.

The training kilometers significantly changed over time ($P_{Time} < 0.001$), but no between-group differences were observed ($P_{Interaction} = 0.85$). Data are presented as mean ± standard error.

Body composition

Total body weight decreased borderline significantly more in the protein group compared with the placebo group (**Table 3**). Whole-body lean mass increased in the protein group as well as in the placebo group following 12 weeks of supplementation (Table 3). The protein group had a larger relative increase in whole-body lean mass than the placebo group (Table 3, **Figure 3**). Truncal lean body mass increased significantly more in the protein group compared with the placebo group ($P_{Interaction} = 0.007$, **Table S1**). Total body fat mass decreased in both groups but significantly more in the protein group compared with the placebo group (Table 3). Furthermore, fat mass/lean body mass ratio was significantly more reduced in the protein group compared to the placebo group (Table 3).

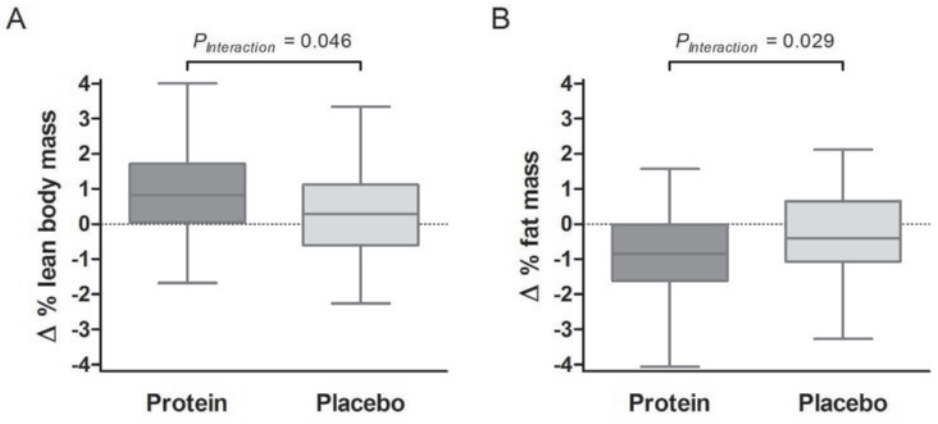


Figure 3. Boxplots showing changes in relative total lean body mass (A) and relative total fat mass (B) in the protein group (dark grey) and placebo group (light grey).

There was a significantly larger increase in relative total lean body mass ($P_{Interaction}$ = 0.046) and a significantly larger decrease in relative total fat mass in the protein group compared with the placebo group ($P_{Interaction}$ = 0.029). Boxplots show the median, upper and lower quartiles and the maximum and minimum values.

Muscle strength and contractile function

Handgrip strength was not improved in both groups after the supplementation period (Table 3). Sub-group measurements of maximal voluntary quadriceps contraction demonstrated also no changes (Table 3). Electrically stimulated quadriceps muscle peak contractions to 1, 10, 30, 50 and 100 Hz for the two groups are shown in **Figure 4** and no between-group differences were observed at baseline or over time. Maximal rate of force rise, early- and half relaxation time were not different between groups over time (Table 3), indicating that no differences occurred in velocity response of the muscle. Muscle fatigue, the significant decline in force of the quadriceps muscle during 2 minutes of electrical stimulation, did not differ between groups at baseline and after the supplementation period (**Figure 5**). Finally, no changes in resistance to fatigue after 2 min of electrical stimulation were found in both groups (Table 3).

Table 3. Changes in body composition, strength, physical performance, blood and urine parameters of participants in the protein and placebo group

	Protein n=58			**Placebo** n=56			**P-value**		
	Pre	Post	Change	Pre	Post	Change	Time	Treatment	Interaction
Body composition									
Body weight, kg	84.59 ± 10.22	84.00 ± 10.28	-0.59 ± 1.41	81.17 ± 10.33	81.02 ± 10.20	-0.15 ± 1.12	**0.003**	0.10	0.07
Lean body mass, kg	56.80 ± 7.97	57.34 ± 8.17	0.54 ± 1.13	56.71 ± 9.35	57.02 ± 9.21	0.31 ± 1.03	**<0.001**	0.90	0.27
Lean body mass, %	66.71 ± 5.93	67.64 ± 5.74	0.93 ± 1.22	68.92 ± 7.08	69.36 ± 7.00	0.44 ± 1.40	**<0.001**	0.11	**0.046**
Fat mass, kg	25.10 ± 6.28	24.20 ± 6.12	-0.90 ± 1.22	22.20 ± 6.19	21.89 ± 6.24	-0.31 ± 1.28	**<0.001**	**0.026**	**0.013**
Fat mass, %	29.39 ± 6.25	28.47 ± 6.12	-0.92 ± 1.19	27.11 ± 7.30	26.72 ± 7.22	-0.39 ± 1.36	**<0.001**	0.11	**0.029**
Ratio fat mass/lean body mass	0.43 ± 0.13	0.41 ± 0.12	-0.02 ± 0.03	0.39 ± 0.15	0.38 ± 0.14	-0.01 ± 0.03	**<0.001**	0.17	**0.032**
Strength									
MVC, N[a]	698 ± 180	706 ± 175	7.2 ± 71.6	691 ± 163	683 ± 163	-8.7 ± 63.1	0.94	0.74	0.38
MVC/kg body weight, N [a]	8.2 ± 1.8	8.4 ± 1.7	0.1 ± 0.9	8.1 ± 1.9	8.3 ± 1.8	0.2 ± 1.6	0.31	0.78	0.84
Maximal rate of force rise, %/ms [b]	1.20 ± 1.13	1.14 ± 0.12	-0.06 ± 0.09	1.21 ± 0.13	1.18 ± 0.14	-0.03 ± 0.11	**0.004**	0.52	0.38
Early relaxation time, ms [c]	27.3 ± 4.3	27.1 ± 4.3	-0.27 ± 3.05	25.9 ± 3.9	26.2 ± 4.1	0.29 ± 2.7	0.98	0.41	0.58
Half relaxation time, ms [d]	35.6 ± 7.1	36.7 ± 9.0	1.1 ± 8.5	37.4 ± 5.6	38.9 ± 6.7	1.5 ± 4.5	0.39	0.47	0.87
Fatigue, % [e]	-30 ± 8	-30 ± 8	-0.6 ± 8.5	-31 ± 10	-30 ± 10	1.1 ± 7.4	0.86	0.85	0.57
Grip strength, kg	37 ± 8	41 ± 9	0 ± 4	38 ± 10	43 ± 11	1 ± 4	0.12	0.37	0.24
Physical performance									
SPPB total, pt	12 (11 – 12)	12 (11 – 12)	0 (0 – 1)	12 (11 – 12)	12 (12 – 12)	0 (0 – 0)	0.10	0.41	0.73
Balance, pt	4 (4 – 4)	4 (4 – 4)	0 (0 – 0)	4 (4 – 4)	4 (4 – 4)	0 (0 – 0)	1.00	0.68	1.00
Gait speed, pt	4 (4 – 4)	4 (4 – 4)	0 (0 – 0)	4 (4 – 4)	4 (4 – 4)	0 (0 – 0)	-	-	-
Gait speed, s	3.2 ± 0.3	2.9 ± 0.4	-0.2 ± 0.5	3.2 ± 0.5	3.0 ± 0.4	-0.2 ± 0.4	**<0.001**	0.72	0.95
Chair-rise, pt	4 (3 – 4)	4 (3.8 – 4)	0 (0 – 1)	4 (3 – 4)	4 (4 – 4)	0 (0 – 0)	0.07	0.25	0.70

Chair-rise, s [f]	10.4 ± 2.2	9.7 ± 2.2	-0.8 ± 2.2	10.2 ± 1.8	9.4 ± 2.3	-0.7 ± 1.9	**<0.001**	0.77	0.86
TUG, s	6.9 ± 0.9	6.5 ± 0.8	-0.4 ± 0.9	6.9 ± 1.2	6.5 ± 1.1	-0.5 ± 0.6	**<0.001**	0.96	0.50
Estimated VO_2max, ml/kg/min [g]	31.1 ± 9.9	34.7 ± 12.1	3.6 ± 7.8	29.5 ± 9.1	32.5 ± 10.6	3.1 ± 6.8	**<0.001**	0.31	0.71
Blood parameters									
25(OH)D, nmol/L [h]	73.7 ± 28.9	93.4 ± 21.1	20.1 ± 19.9	73.8 ± 25.6	96.0 ± 25.8	21.7 ± 16.3	**0.001**	0.76	0.48
Creatine kinase_U/L [h]	148.5 ± 77.1	134.5 ± 76.2	-14.0 ± 57.6	138.2 ± 69.6	140.6 ± 79.9	3.5 ± 57.6	0.34	0.91	0.11
Albumine, g/L [h]	41.3 ± 2.3	41.3 ± 2.3	-0.1 ± 2.1	41.2 ± 2.2	41.6 ± 2.6	0.4 ± 2.1	0.44	0.76	0.20
Creatinine, µmol/L	80.8 ± 16.3	84.7 ± 17.2	3.9 ± 11.0	77.9 ± 13.1	82.8 ± 13.4	4.9 ± 6.8	**<0.001**	0.37	0.56
eGFR, ml/min/1.73m^{-1} [i]	79.4 ± 13.5	78.1 ± 13.4	-1.9 ± 9.8	83.0 ± 9.1	78.7 ± 10.1	-4.1 ± 7.0	**<0.001**	0.42	0.19
Urea, mmol/L [h]	5.9 ± 1.6	8.3 ± 2.3	2.5 ± 1.5	6.1 ± 1.4	6.2 ± 1.2	0.2 ± 1.3	**<0.001**	**0.001**	**<0.001**
Urine parameters									
Albumin / creatinine ratio, mg/mmol [j]	2.7 ± 4.0	2.2 ± 2.3	-0.5 ± 3.3	2.5 ± 4.6	2.5 ± 4.2	-0.4 ± 4.1	0.18	0.84	0.86

Data are presented as mean ± standard deviation or median (interquartile range). Bold values indicate p-value < 0.05. 25(OH)D, 25-hydroxyvitamin D; eGFR, estimated glomerular filtration rate; MVC, maximal voluntary contraction; SPPB, Short Physical Performance Battery; TUG, Timed Up-and-Go; VO_2max, maximal rate of oxygen consumption.

[a] n = 56, [b] n = 44, [c] n = 33, [d] n = 22, [e] n = 30, [f] n = 111, [g] Estimated VO_2max, corrected for age and weight with the Åstrand test (n = 112), [h] n = 113, [i] n = 109, [j] n = 111.

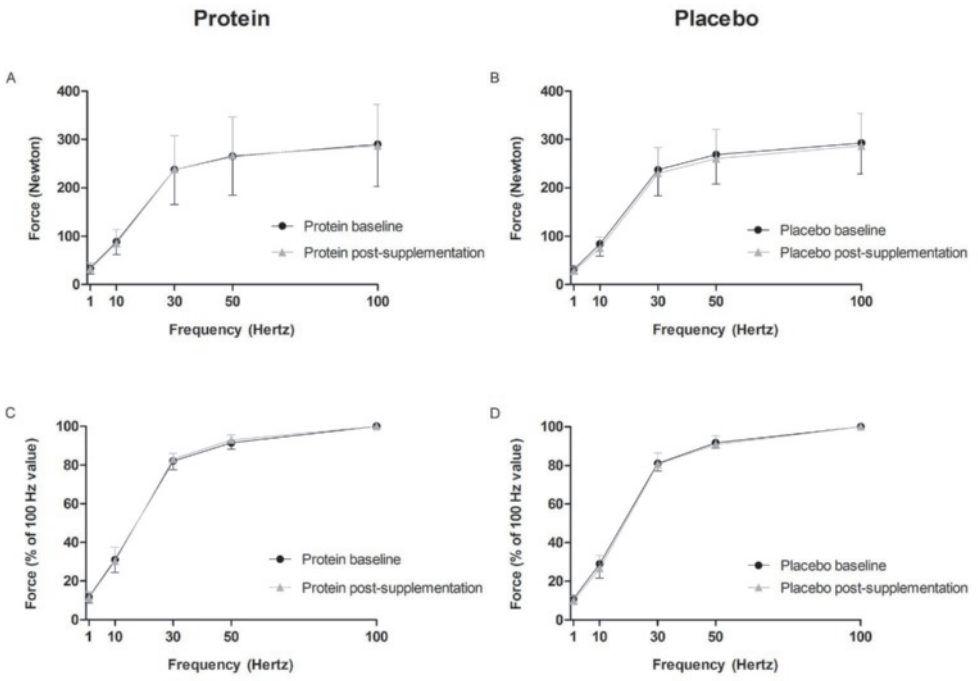


Figure 4. Force responses to different stimulation frequencies (1, 10, 30, 50 and 100 Hz) are given in absolute forces (A and B) and normalized for peak isometric 100-Hz force (relative) (C and D) at baseline and after the supplementation period for the protein group *n* = 20 (A and C) and for the placebo group, *n* = 24 (B and D).

At baseline, the absolute and relative peak forces of the quadriceps were similar between the protein and placebo group ($P_{Interaction}$ = 0.75 and $P_{Interaction}$ = *0.75*, respectively). After the supplementation again no between-group differences were observed in the absolute and relative quadriceps peak forces ($P_{Interaction}$ = 0.33 and $P_{Interaction}$ = 0.20, respectively). Data are presented as mean ± standard deviation.

Physical performance

No significant change in total SPPB score was observed in both the protein and placebo group (Table 3). After 12 weeks, both groups showed faster gait speed (P_{Time} < 0.001), faster chair-rise ability ($PT_{im}e$ < 0.001) faster TUG (P_{Time} < 0.001) and increased estimated VO_2max (P_{Time} < 0.001), but no differences between groups were observed in any of the SPPB subscores, TUG or estimated VO_2max (Table 3).

The effects or 12 weeks of daily protein vs. placebo supplementation on body composition, muscle strength and physical performance are separately given for men and women in **Table S2A** and **S2B**.

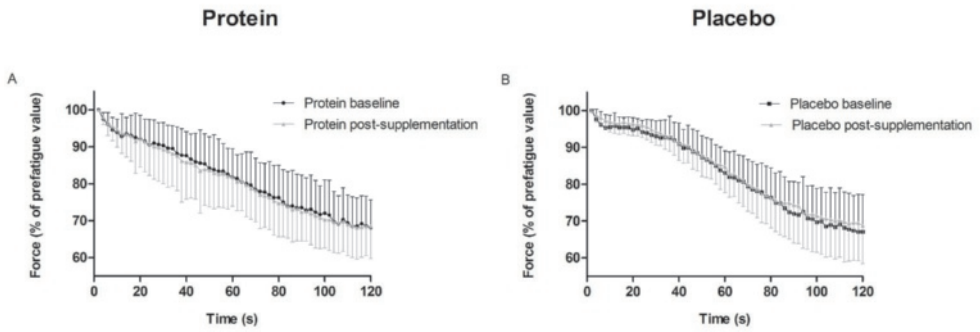


Figure 5. Force responses plotted every second during the fatigue protocol at baseline (t0) and after the supplementation period (t1) for the protein group, *n* = 14 (A) and for the placebo group, *n* = 16 (B).

At baseline the decline in force of the quadriceps was similar between the protein and placebo group ($P_{Interaction}$ = 0.17). For both groups a significant decline in quadriceps force was observed at baseline and after the supplementation (all P_{Time} < 0.001). After the supplementation, again no between-group differences were observed in the decline in quadriceps force ($P_{Interaction}$ = 0.27). Data are presented as mean ± standard deviation.

Biochemical measures

Renal function (eGFR), glucose levels and inflammatory markers (CRP, IL6 and IL10) were similar at baseline (Table 1). At baseline, 79% of the protein group and 84% of the placebo group had a serum 25(OH)D of ≥ 50 nmol/L. The vitamin D status increased in both groups, but no between-group differences were observed (Table 3). In both the protein and placebo group, creatinine concentrations increased and eGFR decreased after 12 weeks, but no between-group differences were observed (Table 3). Serum urea, a breakdown product of protein, increased following 12 weeks of protein supplementation in the protein group, whereas no changes were observed in the placebo group (Table 3). No differences were observed between groups for serum creatine kinase, serum albumin, and urinary albumin/urinary creatinine ratio following 12 weeks of supplementation (Table 3).

DISCUSSION

The present randomized double-blind placebo-controlled trial revealed novel findings about the benefits of 12 weeks protein supplementation in physically active older adults with a low habitual dietary protein intake. First, we found a larger relative increase in lean body mass and a larger decrease in fat mass in the protein intervention group vs. control group. However, no differences in muscle strength, muscle contractile properties, and physical performance were found over time between groups. These findings suggests that age-related loss of muscle

mass can be delayed with an increased protein intake in physically active older adults who have a relatively low habitual protein intake, while no changes were observed in muscle function.

Twelve weeks of protein supplementation induced a relative increase of whole-body lean mass by 0.93 ± 1.22% and a concomitant decrease in fat mass in physically active older adults, which was larger than changes observed in the placebo group. These results are in line with previous studies that investigated the benefits of protein supplementation in frail older adults (30-32), while studies assessing the effect of protein supplementation in community-dwelling older adults found contradicting results. Whereas some studies in community-dwelling older adults found improvements of lean body mass with protein supplementation (33-35), others did not find such beneficial effects (36, 37). A potential explanation for these discrepant findings may relate to differences in the included participants. We specifically selected physically active older adults with a low habitual protein intake based on the FFQ. It has been shown that regular exercise training stimulates muscle protein synthesis, but the muscle protein balance remains negative in the absence of sufficient protein intake (38). Hence, community-dwelling older adults that are not as active as our participants may not benefit from protein supplementation as there is insufficient stimulus for muscle synthesis. Alternatively, we supplemented our physically active participants with 15 g protein at breakfast and 15 g protein after exercise or at lunch, causing a significant increase in daily protein intake from 0.86 ± 0.23 g/kg/d upon enrolment to 1.29 ± 0.28 g/kg/d at 12 weeks. This level of protein intake aligns with guideline recommendations for physically active older adults (6) and seemed sufficient to attenuate the age-induced loss of muscle mass in previous studies (39-41).

The increase in lean body mass and decrease in fat mass was predominantly observed in the trunk. These findings are in alignment with previous studies that revealed an increase in trunk lean body mass following aerobic exercise training, whereas resistance exercise also increased appendicular lean body mass (42, 43). Our participants of both the protein and placebo group mainly performed moderate-intensity walking exercise, which might explain the trunk-specific improvements in both groups. The improvements were however significantly larger in the protein group. Various health benefits have been associated with truncal body composition improvements, such as a reduced risk for cardiovascular diseases and metabolic syndrome (44), improved postural stability, and consequently a reduced risk for falls (45, 46), while the maintenance of lean mass of the trunk may only moderately contribute to the mobility of older adults (47).

We did not find improvements in hand grip strength, nor in quadriceps muscle strength, contractile function and fatigue following protein supplementation. These muscle characteristics all apply to appendicular muscles, while lean body mass mainly increased in the trunk region, which may partly explain the lack of functional improvements seen in these muscles. Lean

body mass improvements are certainly not always accompanied by changes in muscle strength (39), as sometimes, the muscular hypertrophy is not induced by myofibrillar hypertrophy but by sarcoplasmic hypertrophy (48) The latter consists of growth of the sarcoplasm and non-contractile proteins, thus not directly contributing to muscular force (48). Because no biopsies were performed in our volunteers, the identification of the compartment that accumulates proteins cannot be addressed in this study.

While both groups increased their cardiorespiratory fitness, most likely as a result of the increased walking exercise training kilometres, no between-group differences were observed. A previous study showed positive effects of protein supplementation on changes in VO_2max among participants aged 48 ± 7 years (49). However, the participants of the treatment group included in that study were untrained and had lower cardiorespiratory fitness scores at baseline compared with the baseline values of estimated VO_2max of our participants (25.5 ± 4.2 ml/kg/min vs. 31.1 ± 9.9 ml/kg/min, respectively). Untrained participants may benefit more from protein supplementation for improvement of aerobic fitness, than physically active older adults do (50).

Although physical performance as measured with SPPB and TUG improved in both groups after 12 weeks, most likely as a result of the increased walking exercise training kilometres, protein supplementation had no additional impact on these changes. The beneficial effects of a physically active lifestyle might therefore be more pronounced and overrule the benefits of enhancing the protein intake. A study performed in active older men found no additional effect of protein supplementation above the effect of resistance exercise training (51) indicating that the effect of exercise is larger than the effect of protein intake (38). However, the active older men that were studied had an adequate protein intake (1.14 ± 0.05 g/kg/day) already. The results of our study suggest that improving the protein intake in healthy active elderly with an inadequate habitual protein intake can enlarge the health benefits of an active lifestyle by increasing lean body mass. Moreover, it should be noted that the physically active older adults in our study exhibited already a high level of physical performance at baseline (median: 12 (IQR: 11 – 12) with 65% of the participants demonstrating the maximum score of 12 points at baseline) and consequently it was likely that a ceiling effect occurred for most participants. Therefore, SPPB may not be an adequate test in this active group to assess the effect of additional protein supplementation (52). In parallel, the TUG test reports in community-dwelling older adults average scores between 7.9 – 9.0 s (53, 54), whereas our participants already scored 6.9 ± 0.9 s at baseline, thus creating a small window for improvement. Therefore, we should be cautious with our findings that protein supplementation had no effect on physical performance because our tests used may not have been suitable for such an active population. Alternative tests such as 400 m walk test generally give more information in high functioning participants (52) and are recommended to be incorporated in future studies.

The results of the present study indicate that physically active older adults with a low habitual protein intake could gain almost 1% in lean body mass following 12 weeks of protein supplementation of 31 g/day. The average rate of annual loss of muscle mass in older adults is normally approximately 0.5-1.0% (40). Thus, the increase of lean body mass found in our study could be translated into saving 1-2 years of muscle mass decline and is therefore of great significance for daily life mobility on the long-term. The enhanced protein intake did not seem to affect renal function throughout the supplementation period because no differences in eGFR were observed compared with the placebo group and no changes in urinary albumin/urinary creatinine ratio were seen over time. Therefore, enhancing protein intake is not only effective but also a safe strategy (55) to attenuate the age-related loss of muscle mass in physically active older adults.

We performed a double-blinded randomized placebo-controlled trial in a large study population with a low dropout rate and high compliance. However, some limitations should be noted. Our physical performance measurements were most likely not sensitive enough to distinguish improvements between both groups of high-functioning participants. Furthermore, we did not collect 24 h urine in which creatinine could be determined, the gold standard to assess renal function. However, with other parameters such as serum eGFR and urinary albumin/urinary creatinine ratio we were able to determine that renal function was unaffected by the supplementation. We performed explorative sex-specific analyses of our data and found that the beneficial effects of protein supplementation on body composition are more pronounced in women than in men. We acknowledge that our study was not powered for these sub-analyses, but the outcomes suggest that more studies are warranted to assess possible differences between men and women in responses to protein supplementation.

Conclusion

In physically active older adults with relatively low habitual dietary protein consumption an improvement in physical performance, an increase in lean body mass and a decrease in fat mass were observed after walking exercise training. Twelve weeks of protein supplementation resulted in a relative larger increase in lean body mass and a larger decrease in fat mass compared with the placebo group. This was not accompanied by differences in improvements in muscle strength or physical performance between both groups. The improved body composition shows that protein supplementation enlarges the proposed health benefits of an active lifestyle in physically active older adults, but physical performance could not be improved further in already vital older adults.

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SUPPLEMENTAL MATERIAL

Table S1. Changes in regional body composition of participants in the protein and placebo group

	Protein n=58			Placebo n=56			P-value		
	Pre	Post	Change	Pre	Post	Change	Time	Treatment	Interaction
Trunk									
Trunk lean body mass, kg	27.6 ± 3.7	27.9 ± 3.9	0.3 ± 0.9	27.2 ± 4.5	27.3 ± 4.4	0.1 ± 1.0	**0.038**	0.49	**0.007**
Trunk lean body mass, %	63.1 ± 5.9	64.0 ± 5.9	0.9 ± 1.5	65.0 ± 7.3	65.3 ± 7.2	0.3 ± 2.1	**0.001**	0.20	**0.047**
Trunk fat mass, kg	15.4 ± 4.2	14.9 ± 4.2	-0.5 ± 0.9	13.7 ± 4.1	13.7 ± 4.2	0.0 ± 1.0	**0.003**	0.06	**0.011**
Trunk fat mass, %	34.6 ± 6.0	33.6 ± 6.0	-1.0 ± 1.6	32.5 ± 7.3	32.3 ± 7.3	-0.1 ± 2.0	**0.002**	0.17	**0.017**
Arms									
Arm lean body mass, kg	6.0 ± 1.2	6.0 ± 1.2	0.0 ± 0.4	6.0 ± 1.4	6.0 ± 1.3	0.0 ± 0.2	0.89	0.84	0.51
Arm lean body mass, %	67.9 ± 7.2	67.7 ± 6.6	-0.2 ± 1.9	69.6 ± 7.6	69.5 ± 7.3	-0.1 ± 1.8	0.41	0.18	0.78
Arm fat mass, kg	2.4 ± 0.8	2.4 ± 0.7	0.0 ± 0.3	2.1 ± 0.6	2.1 ± 0.6	0.0 ± 0.2	0.54	**0.029**	0.68
Arm fat mass, %	27.1 ± 7.8	27.3 ± 7.1	0.2 ± 2.1	25.3 ± 8.0	25.4 ± 7.7	0.1 ± 1.8	0.47	0.18	0.69
Legs									
Leg lean body mass, kg	19.4 ± 3.1	19.6 ± 3.1	0.2 ± 0.7	19.6 ± 3.5	19.7 ± 3.5	0.2 ± 0.7	**0.003**	0.85	0.51
Leg lean body mass, %	71.1 ± 8.5	72.3 ± 8.3	1.2 ± 1.4	73.6 ± 9.1	74.6 ± 9.1	1.0 ± 1.4	**<0.001**	0.15	0.31
Leg fat mass, kg	6.5 ± 2.6	6.2 ± 2.5	-0.4 ± 0.5	5.6 ± 2.3	5.3 ± 2.3	-0.3 ± 0.4	**<0.001**	**0.049**	0.22
Leg fat mass, %	23.9 ± 9.0	22.7 ± 8.8	-1.3 ± 1.4	21.5 ± 9.5	20.5 ± 9.4	-1.0 ± 1.4	**<0.001**	0.18	0.26

Data are presented as mean ± standard deviation. Bold values indicate p-value < 0.05.

Table S2A. Changes in body composition, strength, physical performance, blood and urine parameters of the male participants in the protein and placebo group

	Protein n=47			Placebo n=46			P-value		
	Pre	Post	Change	Pre	Post	Change	Time	Treatment	Interaction
Body composition									
Body weight, kg	83.40 ± 9.11	85.98 ± 9.03	-0.42 ± 1.41	84.04 ± 8.65	83.80 ± 8.62	-0.25 ± 1.08	**0.013**	0.22	0.51
Lean body mass, kg	59.64 ± 5.43	60.18 ± 5.75	0.54 ± 1.09	60.32 ± 5.29	60.59 ± 5.23	0.26 ± 0.95	**<0.001**	0.63	0.20
Lean body mass, %	68.61 ± 4.48	69.36 ± 4.57	0.75 ± 1.00	71.07 ± 5.40	71.49 ± 5.34	0.42 ± 1.27	**<0.001**	**0.027**	0.16
Fat mass, kg	24.06 ± 5.67	23.36 ± 5.67	-0.7 ± 1.08	21.43 ± 6.20	21.10 ± 6.26	-0.34 ± 1.22	**<0.001**	**0.050**	0.13
Fat mass, %	27.35 ± 4.65	26.62 ± 4.76	-0.74 ± 1.00	24.84 ± 5.51	24.47 ± 5.46	-0.37 ± 1.21	**<0.001**	**0.029**	0.12
Ratio fat mass/lean body mass	0.38 ± 0.09	0.37 ± 0.09	-0.01 ± 0.02	0.34 ± 0.10	0.33 ± 0.10	-0.01 ± 0.02	**<0.001**	**0.033**	0.11
Strength									
Grip strength, kg	43 ± 7	43 ± 7	0 ± 4	46 ± 7	47 ± 8	1 ± 4	**<0.001**	0.07	0.52
Physical performance									
SPPB total, pt	12 (11 – 12)	12 (11 – 12)	0 (0 – 0)	12 (11 – 12)	12 (12 – 12)	0 (0 – 0.3)	**0.047**	0.55	0.63
Balance, pt	4 (4 – 4)	4 (4 – 4)	0 (0 – 0)	4 (4 – 4)	4 (4 – 4)	0 (0 – 0)	0.99	0.42	0.42
Gait speed, pt	4 (4 – 4)	4 (4 – 4)	0 (0 – 0)	4 (4 – 4)	4 (4 – 4)	0 (0 – 0)	-	-	-
Gait speed, s	3.2 ± 0.3	3.0 ± 0.4	-0.2 ± 0.4	3.1 ± 0.4	2.9 ± 0.3	-0.2 ± 0.5	**<0.001**	0.43	0.57
Chair-rise, pt	4 (3 – 4)	4 (3 – 4)	0 (0 – 0)	4 (3 – 4)	4 (4 – 4)	0 (0 – 0)	**0.038**	0.25	0.85
Chair-rise, s [a]	10.4 ± 2.2	9.7 ± 2.1	-0.7 ± 2.2	10.3 ± 1.7	9.3 ± 2.0	-1.0 ± 1.6	**<0.001**	0.59	0.52
TUG, s	7.0 ± 0.9	6.6 ± 0.8	-0.4 ± 1.0	6.7 ± 0.9	6.2 ± 0.8	-0.5 ± 0.7	**<0.001**	0.10	0.58
Estimated VO_2max, ml/kg/min [b]	31.0 ± 10.2	38.9 ± 31.4	7.9 ± 31.2	30.4 ± 9.2	33.4 ± 11.4	3.0 ± 6.9	**<0.001**	0.61	0.77

Data are presented as mean ± standard deviation or median (interquartile range). Bold values indicate p-value < 0.05.

MVC, maximal voluntary contraction; SPPB, Short Physical Performance Battery; TUG, Timed Up-and-Go; VO_2max, maximal rate of oxygen consumption.
[a] n = 82, [b] Estimated VO_2max, corrected for age and weight with the Åstrand test (n = 90).

Table S2B. Changes in body composition, strength, physical performance, blood and urine parameters of the female participants in the protein and placebo group

	Protein n=11			Placebo n=10			P-value		
	Pre	Post	Change	Pre	Post	Change	Time	Treatment	Interaction
Body composition									
Body weight, kg	76.87 ± 11.53	75.57 ± 11.40	-1.30 ± 1.22	68.20 ± 6.76	68.51 ± 6.90	0.31 ± 1.22	0.08	0.07	**0.007**
Lean body mass, kg	44.68 ± 5.18	45.23 ± 5.40	0.54 ± 1.34	40.06 ± 4.45	40.61 ± 4.15	0.54 ± 1.36	0.08	**0.040**	1.00
Lean body mass, %	58.58 ± 4.34	60.29 ± 4.28	1.70 ± 1.73	59.00 ± 5.25	59.52 ± 5.12	0.52 ± 1.99	**0.013**	0.93	0.16
Fat mass, kg	29.58 ± 7.03	27.8 ± 6.91	-1.78 ± 1.43	25.72 ± 5.01	25.52 ± 4.90	-0.20 ± 1.61	**0.007**	0.26	**0.027**
Fat mass, %	38.08 ± 4.58	36.37 ± 4.51	-1.71 ± 1.63	37.56 ± 5.07	37.05 ± 5.01	-0.50 ± 1.98	**0.011**	0.97	0.14
Ratio fat mass/lean body mass	0.62 ± 0.12	0.58 ± 0.11	-0.04 ± 0.04	0.61 ± 0.13	0.60 ± 0.12	-0.01 ± 0.05	**0.012**	0.95	0.15
Strength									
Grip strength, kg	30 ± 6	29 ± 6	-1 ± 4	26 ± 7	26 ± 5	0 ± 2	**0.022**	0.24	0.34
Physical performance									
SPPB total, pt	11 (11 – 12)	12 (11 – 12)	0 (-1 – 1)	12 (12 – 12)	12 (12 – 12)	0 (-1 – 0)	0.95	0.55	0.20
Balance, pt	4 (4 – 4)	4 (4 – 4)	0 (0 – 0)	4 (4 – 4)	4 (4 – 4)	0 (0 – 0)	0.96	0.34	0.34
Gait speed, pt	4 (4 – 4)	4 (4 – 4)	0 (0 – 0)	4 (4 – 4)	4 (4 – 4)	0 (0 – 0)	-	-	-
Gait speed, s	3.3 ± 0.4	2.8 ± 0.5	-0.5 ± 0.6	3.5 ± 0.6	3.3 ± 0.6	-0.3 ± 0.3	**0.001**	0.09	0.25
Chair-rise, pt	4 (3 – 4)	4 (4 – 4)	0 (0 – 1)	4 (3.8 – 4)	4 (3.8 – 4)	0 (-0.3 – 0)	0.96	0.79	0.27
Chair-rise, s [a]	10.2 ± 2.0	9.4 ± 2.7	-1.1 ± 2.0	9.7 ± 2.1	9.9 ± 3.2	0.6 ± 2.6	0.63	0.74	0.15
TUG, s	6.7 ± 0.6	6.4 ± 0.9	-0.3 ± 0.5	7.9 ± 1.8	7.4 ± 1.7	-0.4 ± 0.6	**0.004**	0.06	0.64
Estimated VO_2max, ml/kg/min [b]	30.8 ± 8.9	35.0 ± 14.0	4.2 ± 10.7	25.7 ± 7.4	29 ± 4.8	3.3 ± 6.3	0.07	0.15	0.82

Data are presented as mean ± standard deviation or median (interquartile range). Bold values indicate p-value < 0.05.

MVC, maximal voluntary contraction; SPPB, Short Physical Performance Battery; TUG, Timed Up-and-Go; VO_2max, maximal rate of oxygen consumption.
[a] *n* = 19, [b] Estimated VO_2max, corrected for age and weight with the Åstrand test (*n* = 21).

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CHAPTER 6

Determinants of vitamin D status in physically active elderly in the Netherlands

ABSTRACT

Purpose: Vitamin D deficiencies are common in elderly, which increases the risk for e.g. bone fractures. Identification of determinants of vitamin D status may provide leads for specific deficiency prevention strategies. Although determinants of vitamin D status have been studied in various populations, this has not been examined in elderly that have a physically active lifestyle.

Methods: Vitamin D status of 450 physically active elderly who do not use vitamin D supplements was determined and information on possible determinants (demographic, dietary intake and physical activity) was collected around a prolonged four day walking event in July and analyzed in linear regression models.

Results: The average summertime serum 25(OH)D concentration was 88.8 ± 22.4 nmol/L. Only 2% of the participants had a 25(OH)D concentration below 50 nmol/L. Dietary intake of vitamin D was 4.0 ± 1.9 µg/day, and the participants spent 12.4 ± 8.6 hrs/week on outdoor activities. In the multivariate model lower age (β=-0.48, 95% CI -0.80 – -0.16), lower BMI (β=-0.86, 95% CI -1.62 – -0.10), being a moderate to high drinker *versus* a non-drinker (β=7.97, 95% CI 0.43 – 15.51) and more outdoor physical activity (β=0.25, 95% CI 0.01 – 0.50) were significantly associated with higher 25(OH)D concentrations.

Conclusions: In physically active elderly vitamin D status was very high in summertime, with few deficiencies, suggesting that elderly with a physical active lifestyle might not necessarily need supplements during the summer period. Lower age, lower BMI, higher alcohol intake and more outdoor physical activity had a significant association with vitamin D status.

INTRODUCTION

Vitamin D is an essential micronutrient that has several functions, such as the formation of bone tissue and absorption of calcium from the gastrointestinal tract [1,2]. The most important source of vitamin D is the skin, which can produce vitamin D from 7-dehydrocholesterol during exposure to ultraviolet (UV) radiation [3]. The rate of cutaneous vitamin D synthesis is reduced in elderly, and therefore they are at risk for vitamin D deficiencies [4]. For instance, in the Netherlands, about 50% of community-dwelling elderly has a vitamin D deficiency [5], which has led to standard supplementation guidelines for elderly [6,7,8]. However, blood concentrations of 25-hydroxy vitamin D (25(OH)D), the accepted vitamin D status marker [9], can vary considerably between persons, even between persons that appear to receive the same daily dose of vitamin D [10]. This suggests that other factors affect concentrations of 25(OH) D and that the current generalized vitamin D supplementation practices may be inadequate in certain cases. Moreover, based on the age-dependent decline in cutaneous vitamin D synthesis, it may be expected that vitamin D status is lower in subgroups of higher age, but this has not been demonstrated before. A better understanding of the determinants of vitamin D status is therefore required to improve vitamin D status at both the individual as well as the population level.

In recent years, several publications have aimed to identify potential determinants of vitamin D status, such as use of supplements, age and lifestyle factors [5,11-16]. However, these studies have several limitations, amongst others a limited physical activity range of the participants. Especially, knowledge on vitamin D status and its determinants in physically active elderly is lacking.

In the present study, the vitamin D status is investigated in different age subgroups in physically active elderly aged 65-93 yr who do not use vitamin D supplements. In addition, determinants that contribute to vitamin D status were explored. We hypothesized that vitamin D status is relatively high in physically active elderly, and that dietary intake and outdoor physical activity are significant contributors to vitamin D status.

MATERIALS & METHODS

Study population

Participants of the Four Days Marches of 2015 or 2016, an annual four day walking event in the Netherlands that takes place in July, were recruited via newsletters and internet advertisements. Participants had to be 65 yr or older and caucasian. The study adhered to the Declaration of Helsinki. The Medical Ethical Committee of the Radboud University Medical Center approved

the study (study-id: NL36743.091.11), and all participants gave written informed consent prior to participation.

Study design

During this cross-sectional study, participants filled in two online questionnaires. The first questionnaire assessed demographic characteristics (sex, age, ethnicity, body weight and height and smoking), use of supplements and habitual physical activity levels with the validated SQUASH questionnaire [17]. The second questionnaire was a validated food frequency questionnaire about their habitual dietary intake [18,19]. Furthermore, participants visited our field laboratory at the event location one or two days prior to the first walking day to collect a venous blood sample of 3.5 ml.

Analysis of blood vitamin D concentrations

Venous blood was drawn from the antecubital vein in Vacutainer collection tubes (Becton Dickinson, Vianen, the Netherlands) and was allowed to clot for at least 30 minutes at room temperature. Within 4 hours after collection, the blood was centrifuged and serum was stored at -80 °C until further analysis. Serum 25(OH)D3 concentrations were determined using a commercially available kit with high-performance liquid chromatography coupled to ultraviolet detection (HPLC-UV; Chromsystems Instruments & Chemicals GmbH, Gräfelfing, Germany) for samples collected in 2015 (n=378), or a method using liquid chromatography coupled to tandem mass spectrometry detection (LC-MS/MS; Waters Chromatography B.V., Etten-Leur, the Netherlands) for samples collected in 2016 (n=72). Briefly, both methods consisted of a protein precipitation step and solid phase extraction prior to analysis on the HPLC-UV or LC-MS/MS system. Calibrators from the same source (Chromsystems) were used on both systems. Quality control samples at different concentrations were included in each analytical batch to monitor the quality of the analysis. All analyses were performed in the Clinical Chemistry and Haematology Laboratory of Gelderse Vallei Hospital (Ede, the Netherlands) by trained technicians using standard operating procedures. A previously performed direct comparison of the in-house HPLC and LC-MS/MS methods revealed that 25(OH)D concentrations obtained with the LC-MS/MS method were on average 10% higher than the HPLC method results (internal method validation report, unpublished data); therefore, a correction factor of -10% for the LC-MS/MS values was applied to align the 25(OH)D data prior to further statistical analyses.

Physical activity

Physical activity was assessed by the validated Short Questionnaire to Assess Health enhancing physical activity (SQUASH) [17]. SQUASH estimates habitual physical activity during a normal week over the past month. Questions include the type, duration and frequency of activities. The total amount of physical activity in hours per week (hr/wk) was calculated [20]. Participants

were excluded if questionnaires were incomplete and when the total minutes of activity per day exceeded 960 minutes [17]. We incorporated domestic work activities, leisure time activities and sports to assess activities of daily living (i.e., total physical activity). Individual activities were categorized as ''outdoor'' based on discussion with experts that are familiar with the physical activity habits in the Netherlands. Hours per week spent on outdoor leisure time activities and sports activities were calculated.

Dietary assessment

An online validated 180–item semi–quantitative Food Frequency Questionnaire (FFQ) was used to assess habitual daily energy intake, vitamin D intake and alcohol consumption [18,19]. The FFQ reference period was one month, and portion sizes were estimated using standard portions [21]. Nutritional intake was calculated using the Dutch Food Composition Database of 2010 [22]. Some participants were not able to fill in the online questionnaires and dieticians assessed their daily dietary intake with two 24-hr recalls (n=30). The two days were randomized over the week with the restriction that no participant was assigned two identical week days (e.g. two Mondays) or two weekend days (e.g. Saturday and Sunday). The mean of both days was considered to represent their common eating pattern. Alcohol consumption was derived in gram per day of pure alcohol. Based on the alcoholic one drink-equivalent of 14 g of pure alcohol and the American guidelines [23], we divided the participants into non-drinkers, low drinkers, moderate drinkers and high drinkers. A non-drinker was defined as 0.0 – 2.0 gram of alcohol per day which is equivalent to zero to maximally one drink per week. A low drinker was defined as 2.06 – 20.86 gram for females and 2.06 – 34.86 gram for males, which is equivalent to ≥ 1 glass per week to 1.5 or 2.5 glasses per day for females and males, respectively. A moderate drinker was defined as ≥1.5 glasses to 3.5 glasses per day for females (20.87 – 48.86 gram) and≥2.5 glasses to 4.5 glasses per day for males (34.87 – 63.0 gram). A high drinker was defined as ≥3.5 glasses per day for females (≥ 48.87 gram) and ≥4.5 glasses per day for males (≥ 63.06gram).

Statistical analysis

The statistical analyses were performed using SPSS 22 software (IBM SPSS Statistics for Windows, Version 22 IBM Corp., Armonk, NY, USA), with the level of significance set at $p < 0.05$ (two-sided). Participant characteristics were displayed as means ± SDs or as counts with percentages for categorical variables. The total group was divided in three age groups (65-74 yr, 75-84 yr and 85-93 yr) and differences in serum 25(OH)D concentration and baseline characteristics were analyzed between age groups using one-way ANOVA, and using the Chi-square test or Fisher's exact test for categorical variables. Furthermore, after checking the assumptions for linear multiple regression, the associations between possible determinants (i.e. age, sex, BMI, smoking status, vitamin D intake via nutrition, alcohol intake and physical activity) and serum 25(OH)D concentration (nmol/L) were analyzed univariate and multivariate

with linear regression model (forced entry method). To avoid large discrepancies in subgroup sizes, the moderate and high alcohol intake groups were merged.

RESULTS

Population characteristics

We included 450 physically active elderly between the age of 65 and 93 in the present study (Figure 1, Table 1). Seventy-eight percent of the participants were male, aged 71.9 ± 6.8 yr and with a BMI of 25.0 ± 2.9 kg/m^2. The mean serum 25(OH)D concentration in the summer was 88.8 ± 22.4 nmol/L, and serum 25(OH)D concentrations < 50 nmol/L and < 75 nmol/L were present in 2% and 24% of the population, respectively (Figure 2, Table 1). The mean daily energy intake was 2264 ± 650 kcal for males and 1934 ± 463 kcal for females. The vitamin D intake via nutrition was 4.0 ± 1.9 µg/day, with 99% of the participants having an intake below the generally accepted recommendation of 20 µg/day [1,24]. The participants spent 12.4 ± 8.9 hrs/week on outdoor activities.

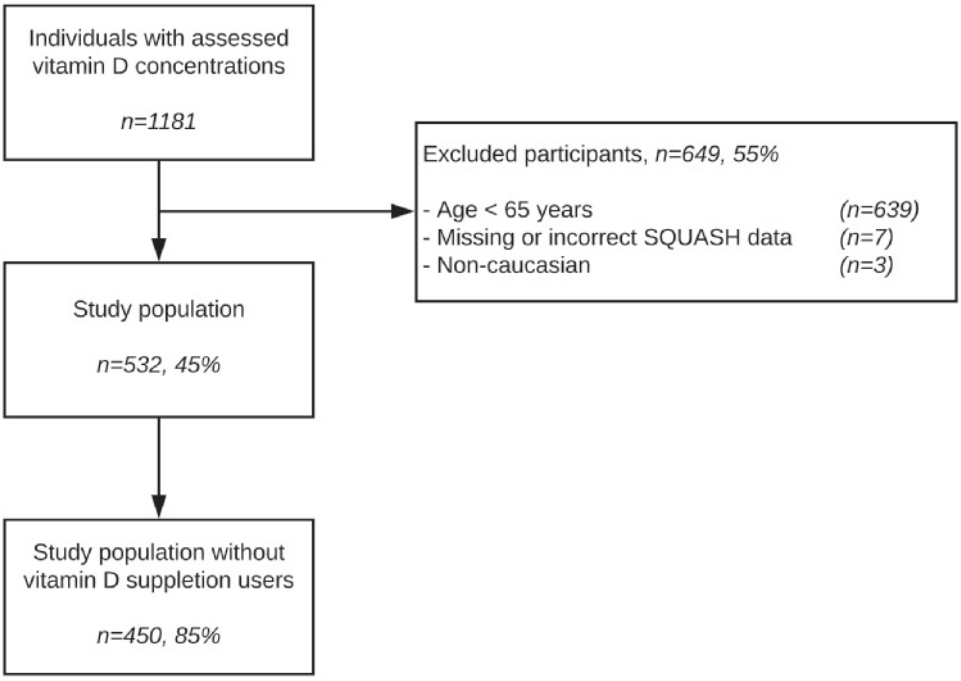

Figure 1. Flowchart for enrollment of the study population

Table 1. Baseline characteristics of the participants that do not use vitamin D suppletion, stratified by 10 yr age groups

Variable	Total *n*=450	65-74 yr *n*=331	75-84 yr *n*=94	85-93 yr *n*=25	*P*-value
Age, yr	71.9 ± 6.8	68.3 ± 2.7	80.6 ± 3.0	87.1 ± 1.9	**<0.001**
Male, n (%)	353 (78)	257 (78)	77 (82)	19 (76)	0.64*
BMI, kg/m2	25.0 ± 2.9	25.1 ± 2.9	24.9 ± 3.0	24.0 ± 2.2	0.13
Currently smoking, n (%)	19 (4)	18 (6)	1 (1)	0 (0)	0.13‡
Vitamin D status					
25(OH)D, nmol/L	88.8 ± 22.4	91.0 ± 23.1	84.1 ± 19.2	77.8 ± 18.6	0.092
25(OH)D ≥ 50 nmol/L, n (%)	441 (98)	324 (98)	92 (98)	25 (100)	**<0.001***
25(OH)D ≥ 75 nmol/L, n (%)	343 (76)	268 (81)	62(66)	13 (52)	**<0.001***
Dietary intake					
Vitamin D via nutrition, µg	4.0 ± 1.9	4.1 ± 1.7	4.1 ± 2.4	3.2 ± 2.7	0.09
Alcohol, g/d	14.4 ± 14.6	15.2 ± 14.8	12.4 ± 14.0	10.7 ± 13.1	0.13
Non-drinker, n (%)	99 (22)	64 (19)	26 (28)	9 (36)	0.14‡
Low drinker, n (%)	289 (64)	223 (67)	53 (56)	13 (52)	
Moderate drinker, n (%)	45 (10)	38 (12)	5 (5)	2 (8)	
High drinker, n (%)	7 (2)	6 (2)	1 (1)	0 (0)	
Total physical activity					
Total physical activities, hr/wk	29.1 ± 16.4	30.4 ± 16.8	25.6 ± 14.3	25.3 ± 15.8	**0.021**
Domestic work activities, hr/wk	10.2 ± 10.7	10.3 ± 10.9	10.0 ± 10.7	8.6 ± 7.5	0.73
Leisure time activities, hr/wk	13.1 ± 9.4	13.6 ± 9.4	11.6 ± 7.8	13.2 ± 13.1	0.20
Sports activities, hr/wk	5.7 ± 6.1	6.3 ± 6.1	4.2 ± 5.1	3.5 ± 7.8	**0.002**
Outdoor physicalactivity					
Total physical activities outdoor, hr/wk	12.4 ± 8.6	12.8 ± 8.8	11.3 ± 7.6	10.4 ± 8.8	0.15
Leisure time activities outdoor, hr/wk	11.0 ± 7.9	11.4 ± 8.1	10.2 ± 7.2	10.2 ± 8.8	0.39
Sports activities outdoor, hr/wk	1.2 ± 2.9	1.3 ± 2.8	1.2 ± 3.4	0.2 ± 0.8	0.16

Data are presented as mean ± SD or number (percentage) of participants. Bold values indicate β with p-value < 0.05.

BMI; body mass index, 25(OH)D; 25-hydroxy vitamin D.

* Derived by Chi-square test. ‡ Derived by Fisher's exact test

Serum 25(OH)D concentrations across 10 yr age groups

Mean serum 25(OH)D concentration were 91.0 ± 23.1 nmol/L, 84.1 ± 19.2 nmol/L and 77.8 ± 18.6 nmol/L for the age groups 65-74 yr, 75-84 yr and 85-93 yr, respectively (Table 1). Although mean 25(OH)D values were not significantly different between the age subgroups, significantly more participants in the 85-93 yr group had a serum 25(OH)D concentration ≥ 50 nmol/L, whereas less participants in this oldest age group had serum 25(OH)D concentration ≥ 75

nmol/L compared to the younger age groups. Moreover, sex, BMI, smoking, vitamin D intake via nutrition and alcohol intake did not differ between the age groups. Total physical activity (hr/wk) was significantly higher in participants aged 65-74 yr versus participants aged 75-84 yr (*P* = 0.037). Participants aged 65-74 yr performed more sports activities compared to participants aged 75-84 yr (*P* = 0.007). Outdoor physical activities were not significantly different between age groups.

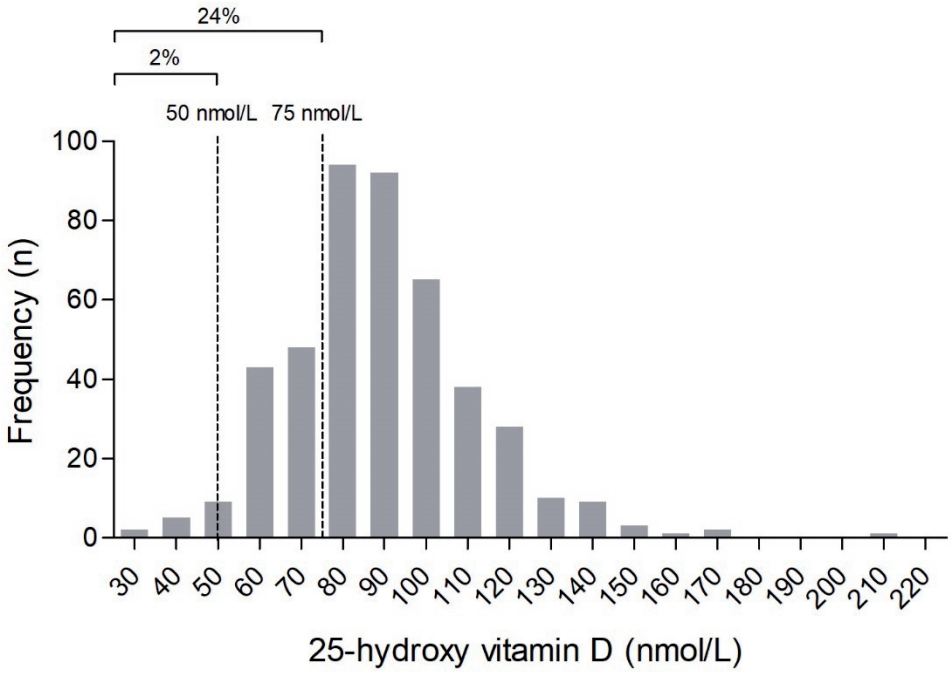

Figure 2. Frequency distribution of 25(OH)D concentrations (nmol/L) of 450 physically active elderly that do not use vitamin D supplements.

Mean 25(OH)D concentrations was 88.8 ± 22.4 nmol/L. A total of 2% were below the threshold for 25(OH)D concentration of 50 nmol/L and 24% were below the 75 nmol/L threshold for 25(OH)D concentration. These findings suggests that elderly who are physically active are able to reach a good vitamin D status, with a low prevalence of deficiencies.

Table 2. Associations between demographic and lifestyle factors (sex, age, BMI, smoking, vitamin D via nutrition, alcohol consumption and physical activity) and 25(OH)D. Data were analyzed using linear regression with 25-hydroxy vitamin D (nmol/L) as the dependent variable.

	25(OH)D, nmol/L	
	Univariate β (95% CI)	**Multivariate β (95% CI)***
Age, yr	**-0.54 (-0.84 – -0.23)**	**-0.48 (-0.80 – -0.16)**
Sex§		
Male (ref)	1.00	1.00
Female	-0.60 (-5.66– 4.46)	-2.39 (-7.83– 3.06)
BMI, kg/m2	-0.70 (-1.43 – 0.03)	**-0.86 (-1.62 – -0.10)**
Smoking§		
Non-smoker (ref)	1.00	1.00
Current smoker	1.34 (-8.96 – 11.65)	-1.24 (-11.49 – 9.02)
Vitamin D via nutrition, µg	0.71 (-0.40 – 1.82)	0.21 (-0.93 – 1.35)
Alcohol§		
Non-drinker (ref)	1.00	1.00
Low drinker	**6.41 (1.49 – 11.33)**	5.10 (-0.15 – 10.36)
Moderate to high drinker	**9.70 (2.33 – 17.08)**	**7.97 (0.43 – 15.51)**
Total physical activities outdoor, hr/wk	**0.28 (0.04 – 0.52)**	**0.25 (0.01 – 0.50)**

*Adjusted for all variables shown in the table. § Categorical variable in which we indicated one option as the constant against which other options were compared.
Bold values indicate β with p-value < 0.05.
BMI; body mass index, 25(OH)D; 25-hydroxy vitamin D.

Determinants of serum 25(OH)D concentration

Lower age (P = 0.001), being a low or moderate to high drinker compared to a non-drinker (P = 0.011, P = 0.010, respectively) and more outdoor physical activity (P = 0.023) were associated with a higher serum 25(OH)D concentration in the univariate analysis, whereas sex, BMI, smoking and dietary vitamin D intake were not associated with serum 25(OH)D concentration (Table 2). In the multivariate model with correction for all variables, lower age (P = 0.003), lower BMI (P = 0.026), being a moderate to high drinker compared to a non-drinker (P = 0.038) and more outdoor physical activity (P = 0.046) were associated with a higher vitamin D status (Table 2). In total, these variables explained 5.9% of the variation of the serum 25(OH)D concentration**.** The assumptions of linear regression were met.

DISCUSSION

In the present study, the vitamin D status and its determinants were investigated in a group of physically active elderly in the summertime. The main findings were that physically active elderly who do not take supplements have high average 25(OH)D blood concentrations in the summer, with only ~2% of the population demonstrating a 25(OH)D concentration< 50 nmol/L. Dietary intake of vitamin D did not significantly contribute to vitamin D status, whereas lower age, lower BMI, higher alcohol intake and more outdoor physical activity were significantly associated with a higher vitamin D status in the multivariate model.

The average vitamin D status of 88.8 nmol/L in elderly aged 65-93 yr, determined in July in the Netherlands, is substantially higher than reported in comparable studies. Brouwer-Brolsma *et al.* investigated vitamin D status in community-dwelling elderly aged ≥ 65 yr, and reported a mean 25(OH)D concentration of 70 nmol/L in blood samples that were collected in July [5]. Moreover, in our population, only 2% had a blood 25(OH)D value of < 50 nmol/L, whereas Brouwer-Brolsma reported that 37% of the population had a blood 25(OH)D value < 50 nmol/L. Furthermore, van Dam *et al.* reported a mean 25(OH)D concentration of 61.3 nmol/L in the summer months with 33.7% < 50 nmol/L in an elderly population with a mean age of 69 yr [12]. The dietary intake of vitamin D in the current study (4.0 ± 1.9 μg/day) is comparable to what is found previously by Brouwer-Brolsma (~ 4.0-4.5 μg/day) [5], and therefore it is unlikely that dietary intake explains the differences in vitamin D status between the study populations. A more plausible explanation for the higher average 25(OH)D concentration in the present study is that our population spent more time on outdoor physical activity. Previous studies have shown that (outdoor) physical activity is associated with a higher vitamin D status [13,15]. In the current study, elderly spent on average 12.4 hrs/week on outdoor activities compared to an average < 7 hrs/week as reported by Van Dam [12]. Therefore, in all age categories (65-74 yr, 75-84 yr and 85-93 yr), the substantially better vitamin D status in physically active elderly may be explained by higher levels of outdoor physical activity. This suggests that despite the age-related lower rate of cutaneous vitamin D synthesis [4], a high level of outdoor physical activity can compensate for this. Another explanation for the high vitamin D status in this population is the relative low BMI. A high BMI and/or adiposity is associated with a lower vitamin D status or response to supplementation, which is explained by volumetric dilution and/or sequestration in the adipose tissue [6,12,25-27]. Our group of physically active elderly had a mean BMI 25.0 ± 2.9 kg/m^2, compared to a mean BMI of 27.5 ± 4.3 kg/m^{2}and 26.8 ± 3.6 kg/m^2 that was reported for Dutch elderly [5]. Possibly, the high level of (outdoor) physical activity may lead to a high vitamin D status through exposure to UV light as well as lowering the BMI.

Generally, elderly are considered a group at risk for vitamin D deficiencies, which has led to generalized vitamin D supplementation guidelines for elderly [2,6]. Although we observed that

significantly more elderly between 65-84 yrs had a 25(OH)D value ≥ 75 nmol/L compared to the 85-93 yr group, the vitamin D status in the entire population is good considering that only 2% of the population had a blood 25(OH)D value < 50 nmol/L. These observations put general vitamin D supplementation guidelines to question, as it shows that physically active elderly seem to reach a sufficient vitamin D status without supplementation, at least in the summertime. It is important to note that we did not measure vitamin D status in winter months. Brouwer-Brolsma investigated the year time fluctuation of vitamin D status in elderly and reported a mean value of ~42 nmol/L in January as the lowest value, and ~70 nmol/L as the highest mean in July [5]. If this finding is extrapolated to our population and 30 nmol/L is subtracted from the summer values, the mean 25(OH)D value would be > 55 nmol/L in the winter, with 34% < 50 nmol/L and 8% < 30 nmol/L. A follow-up evaluation in the winter would be useful to determine to what extent 25(OH)D values will drop in the winter months in physically active elderly who in general remain physically active in winter months as well [5, 12]. The vitamin D status in physically active elderly is high in summertime, which suggests that vitamin D supplementation strategy should take lifestyle factors into account, such as outdoor physical activity, leading to a more personalized and targeted supplementation.

In both the univariate and the multivariate models, age, BMI and outdoor physical activity were associated with 25(OH)D concentrations. These results are in agreement with what has been reported in literature for adults and (community-dwelling) elderly [5,12,15,13,28], where negative associations were found between age, BMI and vitamin D status, and positive associations were found between physical activity and vitamin D status.

To our surprise, alcohol intake appeared as a significant contributor to vitamin D status in the multivariate regression model. A positive association between moderate alcohol consumption and vitamin D status has been reported in the literature before [29]. The average alcohol consumption in our population was 14.4 gr/day, and ranged between zero consumption up to 79.3 gr/day, meaning that the population contained non-drinkers, low, moderate and some high drinkers. Van Grootheest *et al*. observed a positive correlation between both moderate and high alcohol consumption and 25(OH)D blood levels in a healthy adult population in the Netherlands [25]. Similar associations were observed in a German and Finnish population of (elderly) adults [30,31]. These findings have not been discussed extensively and their relevance for humans is as yet not known. It is possible that the association is explained by drinking outdoor rather than the alcohol itself. Considering that alcohol may also be consumed during e.g. diner or later in the evening (when UV-based vitamin D synthesis is no longer active) we believe that *outdoor* drinking certainly not fully explains the association. In addition, literature suggests that alcohol itself may alter vitamin D metabolism. Experiments with female rats have demonstrated that chronic ethanol consumption leads to reduced renal CYP27B1 expression, with subsequent lower concentrations of 1,25-dihydroxy vitamin D (1,25(OH)$_2$D, the active vitamin D metabolite),

and higher 25(OH)D blood concentrations [32]. It is relevant to know whether the same occurs in humans, as this may lead to overestimation of vitamin D status while the levels of the active vitamin D metabolite may in fact be decreased. Thus, more research is needed to determine whether the observed positive association between vitamin D status and alcohol intake in humans can be explained by altered vitamin D metabolism.

A limitation of the current study is that our questionnaire did not specifically determine the level of outdoor physical activity and exposure to UV radiation. However, we included participants who were training for a multi-day long-distance walking event and therefore most physical activity was performed outside. Furthermore, all vitamin D data was collected within 48 hours, which enabled us to assess determinants of vitamin D status without seasonal effects in vitamin D concentrations. A potential problem of this approach is that we assessed vitamin D status in summer only, and we do not know to what extent these values decrease in winter months.

In conclusion, this study demonstrates that physically active elderly without any supplements have a good vitamin D status in the summer with a low prevalence of deficiencies. From the explored potential determinants of vitamin D status, age, BMI, alcohol intake, and outdoor physical activity contributed significantly to vitamin D status. This report shows that current generalized supplementation recommendations for elderly might lead to unnecessary supplementation in physically active subpopulations in the summer. More research is needed to understand the observed association between alcohol intake and vitamin D status.

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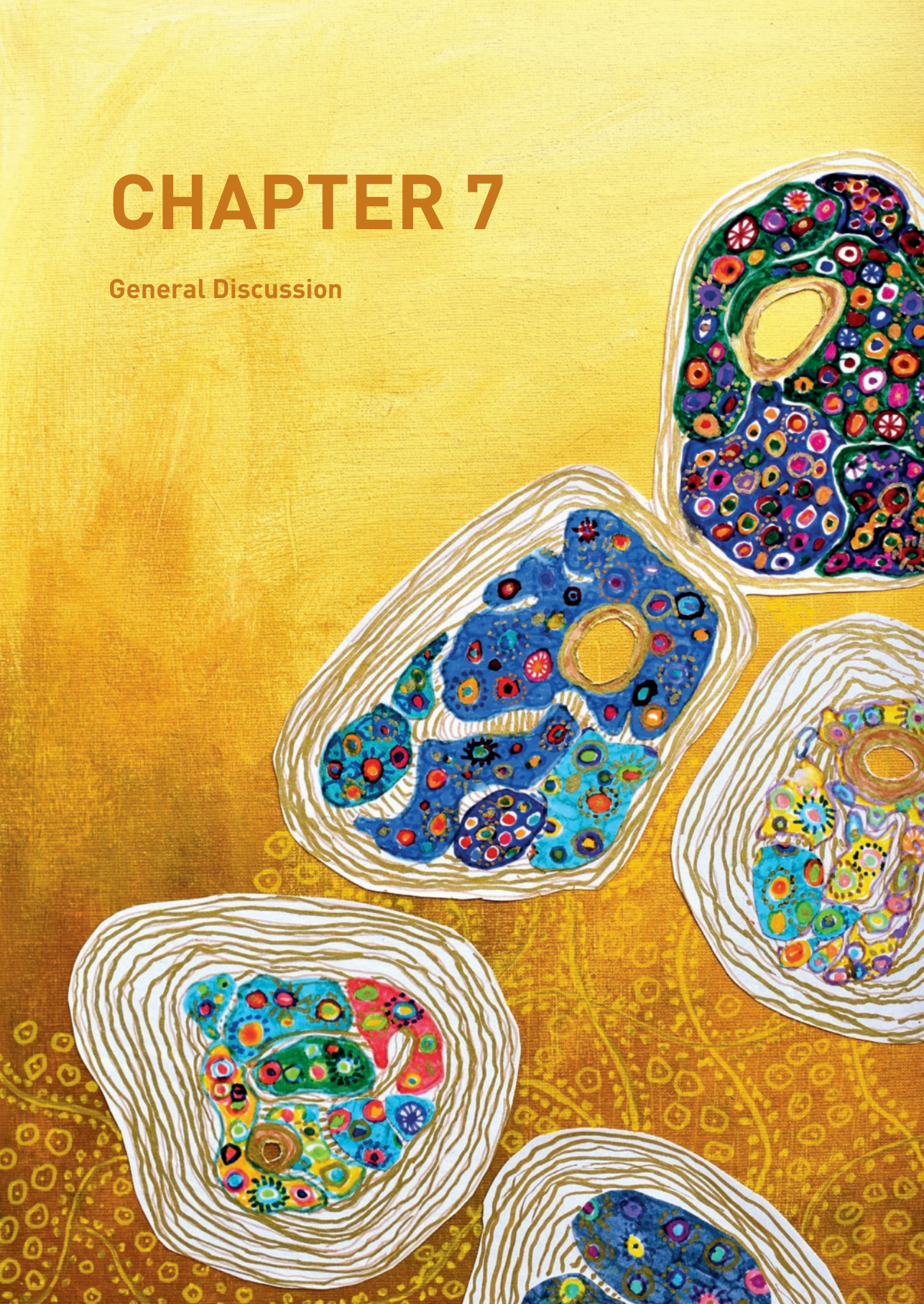
CHAPTER 7

General Discussion



Preservation of muscle mass, strength and physical function during ageing are critical prerequisites to preserve mobility and independence for daily activities. Currently, most research is focused on counteracting the loss of muscle mass and function in (pre-)frail older adults, despite the emerging subpopulation of vital, physically active older adults. Although physically active people often have higher absolute muscle mass and strength compared to their inactive and (pre-)frail peers, they experience age-related declines in muscle mass and strength as well [1, 2]. Since prevention is preferred above treatment, we assessed in this thesis whether habitual protein intake and protein supplementation could prevent or delay the loss of muscle mass and function in physically active older adults. In this final chapter, we elaborate on our findings in a broader perspective and suggest how our results can be translated into practice.

Protein in physically active older adults

Dietary protein intake stimulates skeletal muscle protein synthesis and inhibits protein breakdown, resulting in a positive protein balance [3] and is thus important for the maintenance of muscle mass, strength and function. While the recommended daily allowance (RDA) for protein for adults is 0.8 gram per kilogram body weight per day (g/kg/d), it has been suggested that this RDA for protein intake may not be adequate for older adults, because they have an attenuated capacity of protein utilization for muscle protein synthesis [4]. The PROT-AGE study group suggested that older adults above 65 years of age should consume at least 1.0 g/kg/d [4]. Moreover, physically active older adults should consume even more, i.e., ≥1.2 g protein/kg/d to comply with the synergistic effects of exercise and protein intake on muscle protein synthesis [4]. In **chapter 2** we showed that 16% of physically active males above 65 years of age and 10% of the female adults had a daily protein intake below the current protein RDA of 0.8 g/kg/d. Moreover, 42% and 67% of the male older adults and 34% and 56% of the female older adults did not meet the proposed recommended protein intake of 1.0 and 1.2 g/kg/d, respectively. Furthermore, we showed in **chapter 5** that an increase in protein intake from 0.86 ± 0.23 g/kg/d to 1.29 ± 0.28 g/kg/d (mean ± SD) after 12 weeks of milk protein supplementation in physically active older adults, was associated with a 0.93 ± 1.22% (mean ± SD) increase in lean body mass. These findings reinforce the hypothesis that it is beneficial for physically active older adults to have a higher protein intake than 0.8 g/kg/d, as endorsed by the PROT-AGE study group. On the contrary, our meta-analysis (**chapter 3**) demonstrates that non-frail community-dwelling older adults do not benefit from enhancing their protein intake, as we found no effects of protein supplementation on lean body mass, muscle cross-sectional area, muscle strength or physical performance. The discrepancy between our meta-analysis and our randomized placebo-controlled trial might be caused by 3 factors:

First, we included only physically active older adults in our randomized placebo-controlled trial who exercised for median: 117 (interquartile range: 81.7 – 173.5) METhr/wk. Physical

activity stimulates both muscle protein synthesis and (to a smaller extent) protein breakdown [5]. The exercise-induced enhanced muscle synthetic response might be more optimally utilized for muscle synthesis when the protein intake is optimized. Only limited information was reported about the physical activity habits of the study participants included in the meta-analysis. Across the Western society, as much as 31-69% of older adults does not meet the physical activity guidelines [6, 7]. Hence, participants included in the meta-analysis are likely to be less physically active compared to our RCT and less synergistic effects of exercise and protein on muscle protein synthesis might have been present.

Secondly, habitual protein intake of most study participants in the meta-analysis were already sufficient (often ≥1.0 g/kg/d), while we only included participants with a habitual protein intake <1.0 g/kg/d in the randomized placebo-controlled trial. By inclusion of participants with a low habitual protein intake in our RCT, we created a large window for possible improvements. To further substantiate this hypothesis, we performed additional analysis on our data described in **chapter 5**. Within our group of physically active older adults with a habitual protein intake of <1.0 g/kg/d upon enrollment, participants with a habitual protein intake <0.8 g/kg/d at baseline (*n* = 24, 41%) showed a significantly larger increase in relative lean body mass after 12 weeks of protein supplementation compared to the participants with a protein intake between 0.8-1.0 g/kg/d (*n* = 34, 59%) (mean ± SEM: Δ1.35 ± 1.19% *versus* Δ0.64 ± 1.16%, respectively, *P* = 0.028, **Figure 1**). Relative fat mass also decreased more after 12 weeks of protein supplementation in the group with a habitual protein intake <0.8 g/kg/d compared to the participants with a protein intake above 0.8 g/kg/d (mean ± SEM: Δ-1.34 ± 0.24% *versus* Δ-0.63 ± 0.19%, respectively, *P* = 0.023, Figure 1). For the subgroup of physically active older adults with a protein intake <0.8 g/kg/d an enhanced protein intake might be especially important, because of the combination of a low protein intake with high physical activity levels. As discussed, not only muscle protein synthesis is stimulated by physical activity, but physical activity also induces (to a smaller extent) muscle protein breakdown [5]. The participants in **chapter 5** often performed prolonged moderate-intensity walking exercise, which might result in a negative muscle protein balance post-exercise when not enough amino acids are provided to the muscle. With another anabolic stimuli, i.e. by enhancing the protein intake [8], this balance may have shifted from muscle protein breakdown to muscle protein synthesis.

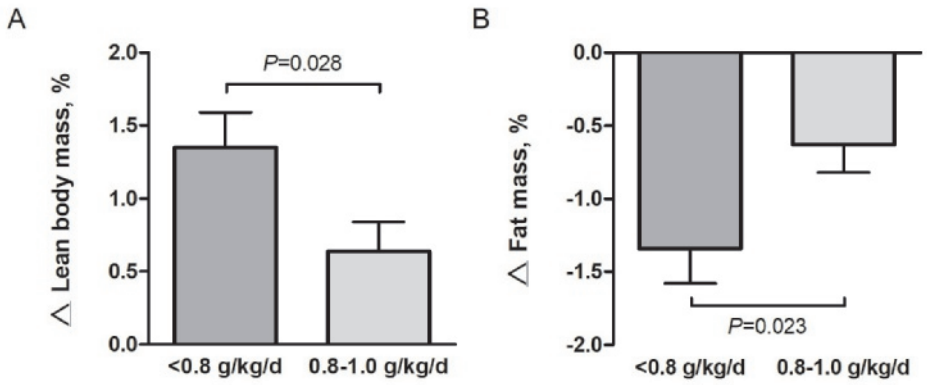


Figure 1. Bar graphs showing changes (mean ± SEM) in relative lean body mass (A) and relative total fat mass (B) after protein supplementation between participants with a habitual protein intake <0.8 g/kg/d (*n* = 24, 41%) and participants with a habitual protein intake between 0.8 – 1.0 g/kg/d (*n* = 34, 59%) upon enrollment.

The increase in relative lean body mass and decrease in relative fat mass after 12 weeks of protein supplementation is larger in the group with a habitual protein intake <0.8 g/kg/d compared to participants with a protein intake between 0.8-1.0 g/kg/d (lean body mass: Δ1.35 ± 1.19% *versus* Δ0.64 ± 1.16%, respectively, *P* = 0.028; fat mass: Δ-1.34 ± 0.24% *versus* Δ-0.63 ± 0.19%, respectively, *P* = 0.023).

Thirdly, protein supplementation strategies in the meta-analysis were often not specifically focused on flaws in the habitual diets of the participants, while in our randomized placebo-controlled clinical trial the protein strategy was aimed on improving meals in which participants had a low habitual protein intake. We found that the habitual protein intake of (physically active) community-dwelling older adults is especially low during breakfast and lunch (**chapter 4 and 5**). The participants in our trial consumed on average 12 ± 6 gram (mean ± SD) of protein during breakfast upon enrollment. This is in agreement with previous literature showing that protein intake during breakfast in community-dwelling, frail and institutionalized older adults does not reach 20 gram [9]. During lunch, protein intake was also low in the frail and institutionalized older adults [9]. In this same study the community-dwelling older adults consumed 27 ± 15 gram (mean ± SD) of protein at lunch [9], whereas the community-dwelling participants in our clinical trial consumed an amount of 20 ± 9 gram (mean ± SD) of protein during lunch. Incorporating doses of 25-30 gram protein in the diet of older adults has been suggested to be a promising strategy to counteract the attenuated post-prandial muscle protein synthesis [10, 11]. To make sure the study participants in our trial reached the amount of 25 gram of protein at breakfast and lunch, we instructed them to consume 1 beverage containing 15 gram of protein during breakfast and the other beverage during lunch. An exception was made for days on which they exercised, then participants were instructed to consume the second beverage within 30 minutes after exercise (*e.g.* walking) because of the exercise-

induced muscle protein synthetic response and the enhanced preservation of skeletal muscle sensitivity to dietary amino acids after exercise [12-14]. This strategy of improving the protein intake during breakfast and lunch has also shown promising results in two other studies performed in non-frail community-dwelling older adults. They found beneficial effects of milk protein supplementation or a milk protein containing product (ricotta cheese) on (appendicular skeletal) muscle mass and physical performance [15, 16]. Many studies in the meta-analysis did not use a protein supplementation strategy focused on compensating the meals with a low protein content, which could have contributed to the lack of positive findings in the meta-analysis. The protein supplementation strategy focused on compensating the shortcomings in protein in breakfast and lunch did result in a significant increase in relative lean body mass in our clinical trial.

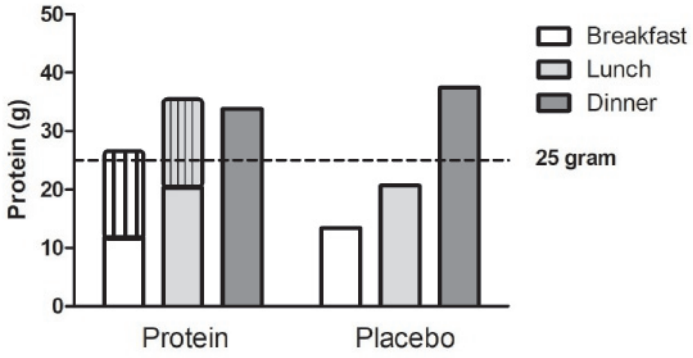


Figure 2. Habitual protein intake during main meals including the protein intake of the supplements of the study participants of our randomized placebo-controlled trial.

The habitual protein intake upon enrollment of the study in the protein group (*n* = 58) was on average 11, 22 and 31 gram during breakfast, lunch and dinner, respectively. We measured the protein intake again after 12 weeks and with the supplement it increased during breakfast to 27 gram as shown with the thick-striped block and therefore met the lower limit of 25 gram as suggested to be necessary to counteract the attenuated post-prandial muscle protein synthetic response in older adults. Moreover, on non-exercising days the protein intake during lunch increased to 35 gram as shown with the thin-striped block. The habitual protein intake upon enrollment in the placebo group (*n* = 56) was 13, 21 and 37 gram during breakfast, lunch and dinner, respectively and thus did not meet the protein requirement of 25 gram during breakfast and lunch.

In conclusion, our data support the recommendation of the PROT-AGE study group that physically active older adults should consume ≥1.2 g protein/kg/d with ≥25 gram of protein per main meal, as we have shown that lean body mass increases and fat mass decreases when physically active older adults meet this recommendation. This improved body composition

could result in multiple health benefits, such as a delay or prevention of the onset of sarcopenia and reduce the risk for cardiovascular and metabolic diseases [17].

Vitamin D status and muscle mass

Whereas about half of the community-dwelling older adults in the Netherlands has a vitamin D status (25(OH)D) below 50 nmol/L [18, 19], rather high average serum 25(OH)D concentration were found in the physically active older adults of our studies. In our trial described in chapter 5, the average 25(OH)D concentration of 114 physically active older adults was mean ± SD: 73.7 ± 27.2 nmol/L in April and these values increased to 96.9 ± 33.8 nmol/L in July. Moreover, in chapter 7 we assessed the vitamin D status of 450 physically active older adults that did not use vitamin D supplements and it was 88.8 ± 22.4 nmol/L in July. It has been proposed that a sufficient vitamin D status may be required to stimulate muscle accretion. This was first suggested when several cross-sectional studies found a positive association between vitamin D status and muscle characteristics [20-22]. The prognostic value of a low serum 25(OH)D concentration for sarcopenia in older persons was also confirmed using a longitudinal design [23]. Therefore, several clinical trials have been designed to investigate the effect of vitamin D supplementation on muscle characteristics in older adults. In a meta-analysis, the results have been pooled and the authors concluded that there was a beneficial effect of vitamin D supplementation on strength and balance [24]. Moreover, a sufficient vitamin D status might positively influence the effect of a nutritional intervention on muscle characteristics. Participants from the PROVIDE study with baseline (25(OH)D concentrations ≥50 nmol/L had greater gains in appendicular muscle mass in response to a nutritional intervention of a vitamin D and leucine enriched whey protein drink compared to participants with insufficient 25(OH)D concentrations [25]. In a post-hoc analysis of our trial described in chapter 5, we found no larger increase in relative lean body mass following a 12-week protein supplementation intervention in participants with a sufficient vitamin D status (25(OH)D concentration ≥50 nmol/L, n = 46) at baseline compared to participants with 25(OH)D concentrations <50 nmol/L, n = 12 (mean ± SD: 0.89 ± 1.17 nmol/L versus 1.11 ± 1.42 nmol/L, P = 0.58, respectively). The average 25(OH)D concentration in the group that received the protein supplementation (n = 58) was 73.7 ± 28.9 nmol/L (mean ± SD) in April. Only 12 people had an insufficient vitamin D status (<50 nmol/L) of which only 3 participants had a vitamin D status <40 nmol/L. In July no participant of the protein group had a vitamin D status < 50 nmol/L. Therefore, as only a few people had a low vitamin D status we were unlikely to detect large confounding effects of a low vitamin D status on muscle characteristics.

Methodological considerations

Across the different studies of this thesis, some generic methodological issues and considerations regarding the measurement of body composition, physical performance and nutritional intake emerged that need to be discussed.

DXA results: absolute versus relative results

Dual-energy X-ray absorptiometry (DXA) is a safe and easy to use measure which provides precise quantification of fat mass, lean body mass and bone mass. Therefore, it is often used to assess changes in body composition in intervention trials as became evident in our meta-analysis described in **chapter 3**. Most studies described in this meta-analysis looked at absolute changes in lean body mass to set conclusions about the effect of their intervention. During our clinical trial described in **chapter 5** we also looked at absolute changes in lean body mass but noticed that weight changes (mostly weight loss) occurred during our 12 week intervention period from April to July. The lost weight consisted mostly of fat mass and not lean body mass, which might have been induced by changes in their nutritional intake and/or increased physical activity during this spring-summer period as a training for the Four Days Marches event (sum of walking kilometers during the 12 week of the study: median: 376 (interquartile range: 255 – 508) km). As the total body weight change was borderline significantly larger in the protein group compared to the placebo group (mean ± SD: -0.59 ± 1.41 kg, -0.15 ± 1.12 kg, respectively, $P = 0.07$), the positive effects of the intervention on the whole body fat mass/lean body mass ratio might have been underestimated. Indeed, while absolute changes in lean body mass were not significantly different between groups, the relative increase in lean body mass of the participants using protein supplements for 12 weeks was significantly larger compared to the control group. Studies assessing the effect of protein supplementation on body composition often have a duration of 12 weeks of longer. Weight changes can easily occur within this time period, especially with seasonal changes and differences might occur between groups. Moreover, we believe the fat mass/lean body mass ratio gives more information about the health status than the absolute numbers of lean body mass or fat mass. Thus, we believe future research should assess relative changes in lean body mass and fat mass as well as absolute changes to prevent underestimation of their results and at the same time give more information about the (changed) health status of their participants.

Physical performance tests in vital, physically active older adults

In the studies described in chapter 4 and 5, the Short Physical Performance Battery (SPPB) and the Timed Up-and-Go test (TUG) were used to assess physical performance. Within our group of vital, physically active, older adults, we experienced that most participants reached high scores for the TUG test and maximal scores for the SPPB. Therefore, we propose that these tests are not suitable for vital physically active older adults as they were not sensitive enough to assess differences between and within participants, whereas other studies in (pre-)frail older adults could use these tests to measure changes in physical performance [26, 27]. While the SPPB can be recommended in terms of validity and reliability, it is prone to a ceiling effect [28]. The SPPB consists of a balance, gait speed and chair-rise test. For gait speed longer distances eliminate the potential ceiling effect in high-functioning older adults [29]. Moreover, in chair-rise tests, standardizing the time (such as 30 sec) instead of the number of required repetitions

on a chair-stand test, improves the test's range and discrimination ability [30]. In the meta-analysis described in chapter 3 it is already shown that there are many physical performance tests which could be used to detect changes in physical performance. We recommend future studies to use the 400 m walk test, 6 min walk test and/or the 30 sec sit-to-stand test to assess changes in physical performance among cohorts of vital physically active, older adults.

Assessing dietary intake: food frequency questionnaire (FFQ) versus 24hr recall

Within this thesis two methods were used to assess dietary intake and specifically protein intake: the food frequency questionnaire (FFQ) and the 24hr recall. In a FFQ, participants report the frequency of consumption and portion sizes of a finite list of food items over a specific period of time in the recent past, in our case, the previous month. In a 24hr recall participants are asked to report in detail their dietary intake of the previous day. The 24hr recall was performed two times on non-consecutive days, which results in a validated average habitual (protein) intake [31]. While the FFQ is easy to fill out for participants independently, the 24hr recall is more extensive and to make sure details are also correctly reported often help of dieticians is necessary. On the other hand, the FFQ does not give information about the distribution of the protein intake over a day, while the 24hr recall does. Thus, while for large groups the FFQ is easy to use and good to estimate protein intake compared to 24hr recalls [32], a 24hr recall might be preferred in smaller sample sizes as it also gives information about the protein distribution. Therefore, after we assessed in **chapter 2** that protein intake was too low in physically active older adults, we used the FFQ only as a screening tool in **chapter 5** and 24hr recalls **in chapter 4 and 5** to determine the protein intake of participants more specifically.

Overall conclusions and translation to daily practice

The work described in this thesis showcases the benefits of a protein intake of ≥1.2 g protein/kg/d with ≥25 gram of protein per main meal in physically active older adults as it might delay or prevent the onset of sarcopenia. While the compliance with our intervention was really high as described in **chapter 5** (mean ± SD: 95 ± 3%), it is difficult for older adults to improve their protein intake by adjusting their own dietary intake. We determined this by performing two 24hr recalls a year after the randomized placebo-controlled trial was performed in 100 participants (April 2017: 0.88 ± 0.21 g/kg/d, Spring 2018: 0.85 ± 0.23 g/kg/d (mean ± SEM), P = 0.19, **Figure 3**). These data suggests that the knowledge that an enhanced protein intake could improve their muscle mass, is not enough for older adults to actively change their habitual protein intake.

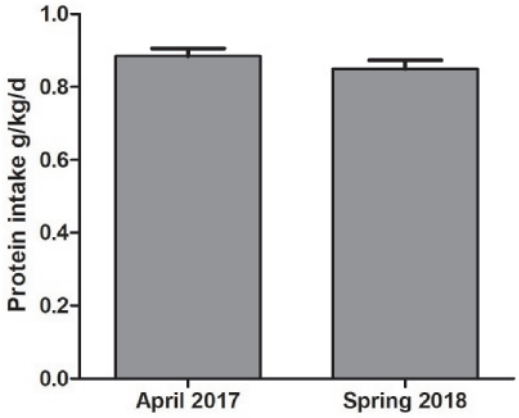


Figure 3. Habitual protein intake (g/kg/d) of 100 participants of the randomized placebo-controlled trial at the start of the study and a year later.

At the start of the study (April 2017) protein intake was 0.88 ± 0.21 g/kg/d and a year later (Spring 2018) protein intake was 0.85 ± 0.23 g/kg/d (mean ± SEM), P = 0.19. Thus, no improvements were seen in habitual protein intake.

Dieticians can give tailored advice and help people make improvements in each individuals diet so that people can maintain these improvements on the long-term. The government of the Netherlands recently decided that from January 1st 2019 more money should be available for prevention in basic care by improving their lifestyle (including behavioral nutrition and physical activity). However, this so-called ¨combined lifestyle intervention¨, *Dutch: gecombineerde leefstijlinterventie (GLI)*, is mainly focused on health risks by overweight [33]. With the increased life expectancy [34] more people will become prone to frailty. Therefore, more attention should be paid to optimizing the protein intake in the growing group of vital and physically active elderly. We advise the government to enable older adults to receive help from dieticians, by which they can improve their protein intake and reduce the risk for sarcopenia and the related health issues of sarcopenia.

Another strategy to enhance protein intake could be the use of a self-assessment application, easy to use for the older population, that determines the protein intake in the habitual diet which gives direct feedback on how people can enhance their protein intake, but also improve their protein distribution with attention for the ratio animal/plant-based proteins. Many older adults are not even aware that their protein intake is low and push-messages might help enhancing their protein intake and distribution.

Moreover, a more practical alternative for older adults might be launching (more) innovative protein enriched products and make these better accessible for the consumer by selling these products in, for example, supermarkets. It has been shown that protein enriched ''regular'' products, such as protein enriched soup, bread or yoghurt, effectively increase the protein intake and improve the protein distribution over the day of older adults in a rehabilitation center and a hospital [35, 36]. We propose these protein enriched products should become available for community-dwelling older adults to prevent or delay the loss of muscle mass and function in community-dwelling older adults.

The mentioned strategies above invoke awareness, initiative and dedication from the older adults themselves. Therefore, it is important that older adults are provided with the knowledge of the importance of this matter. This is why we should keep addressing to the older generation that they can live a longer, healthier and happier life when they are physically active and eat enough proteins.

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CHAPTER 8

Summary
Samenvatting
Datamanagement
Dankwoord
List of publications
Curriculum Vitae
RIHS portfolio

SUMMARY

During ageing a progressive loss in muscle mass occurs which can compromise functional abilities of older adults. In **chapter 1** we introduced this so-called sarcopenia and described several interventions that may prevent or slow down this sarcopenic muscle loss. First, we stressed the importance of physical activity to ameliorate the age-related muscle loss. Another strategy is consuming sufficient protein. In **chapter 2** we investigated whether physically active older adults consume sufficient protein intake. We demonstrated that many physically active older adults do not reach the protein recommendations which may offset the health benefits of an active lifestyle on muscle synthesis.

Increasing the protein intake has shown conflicting results on muscle characteristics, which may partly be explained by the simultaneous inclusion of studies with frail and non-frail older adults. In **chapter 3** we determined, using a meta-analysis, that protein supplementation in exclusively non-frail community-dwelling older adults, does not lead to increases in lean body mass, muscle strength and physical performance, nor does it exert superior effects when added to resistance exercise training. Habitual protein intake was often already sufficient in these older adults. Moreover, habitual physical activity was mostly not reported in the included studies whereas this could influence the results.

In **chapter 4** we assessed whether protein intake and protein intake distribution were associated with muscle strength, physical performance and quality of life in community-dwelling older adults with a wide range of physical activity. While a higher protein intake was not associated with improved physical outcome measures, the combination of a higher protein intake and physical activity was related to better quality of life. Moreover, a more spread protein intake during the main meals was related to higher gait speed. This suggests that a spread protein intake distribution might also be beneficial for physical performance.

In **chapter 5** we combined this knowledge in a randomized placebo-controlled trial using a 12-week protein supplementation intervention protocol for physically active older adults. With this protein supplementation average protein intake increased from 0.86 ± 0.23 g/kg/d to 1.29 ± 0.28 g/kg/d and resulted in a more spread protein intake distribution. We found a larger increase in relative lean body mass after using the protein supplementation when compared to the participants that received placebo supplementation.

In **chapter 6** we showed that the vitamin D status in physically active older adults was very high, without using vitamin D supplements. This suggests that older adults with a physically active lifestyle, mainly based on walking, often have an adequate vitamin D status which might be beneficial because of the proposed positive effects of vitamin D on muscle accretion.

In **chapter 7** we discussed the results of the studies presented in this manuscript. We concluded that physically active community-dwelling older adults benefit from increasing their protein intake by enhancing their relative lean body mass, especially when their habitual protein intake is low. Our data supports the protein recommendation of the PROT-AGE study group for physically active community-dwelling older adults of ≥1.2 g/kg/d. We speculate that regular feedback from a dietician, a self-assessment tool and protein enriched products in the supermarkets can give more awareness and might help improving the habitual protein intake. That way, vital community-dwelling older adults might be able to live a longer and healthier life, independently.

SAMENVATTING

Ouder worden gaat gepaard met verlies van spiermassa, wat zorgt voor fysieke beperkingen en een verlies aan functionele capaciteit. In **hoofdstuk 1** introduceren we deze zogenaamde sarcopenie en beschrijven we diverse interventies die mogelijk het verlies aan spiermassa tegen kunnen gaan. Allereerst benoemen we het belang van fysieke activiteit om het leeftijdgerelateerde spiermassaverlies te verminderen. Daarnaast is het consumeren van voldoende eiwit een mogelijke strategie om het spiermassaverlies tegen te gaan. In **hoofdstuk 2** hebben we onderzocht of fysiek actieve ouderen voldoende eiwitten consumeren. We toonden aan dat veel fysiek actieve ouderen de eiwitaanbevelingen niet halen. Deze onvoldoende eiwitinname kan mogelijk de gezondheidsvoordelen van een actieve levensstijl op spieraanmaak reduceren.

Het verhogen van de eiwitinname heeft tegenstrijdige resultaten laten zien op spiermassa (wat vaak wordt gemeten als vetvrije massa), spierkracht en fysiek functioneren, die mogelijk verklaard kunnen worden door de gelijktijdige inclusie van studies met fragiele ouderen én non-fragiele ouderen. In **hoofdstuk 3** concludeerden wij aan de hand van een meta-analyse dat eiwitsuppletie bij exclusief non-fragiele thuiswonende ouderen niet tot een verbetering van vetvrije massa, spierkracht en fysiek functioneren leidt. Eiwitsuppletie vertoont ook geen additionele positieve effecten op vetvrije massa, spierkracht en fysiek functioneren als het wordt gecombineerd met krachttraining. Vaak was de gebruikelijke eiwitinname van deze ouderen al voldoende. Bovendien werd het beweegpatroon van deze deelnemers vaak niet gerapporteerd in de geïncludeerde studies, terwijl dit wel invloed kan hebben op de resultaten. In **hoofdstuk 4** bepaalden we of eiwitinname en de eiwitverdeling over de dag geassocieerd waren met spierkracht, fysiek functioneren en kwaliteit van leven in thuiswonende ouderen met een gevarieerde fysiek actieve levensstijl. Terwijl een hogere eiwitinname niet geassocieerd was met een verbeterde fysieke functionaliteit, was de combinatie van een hogere eiwitinname en meer fysieke activiteit wel gerelateerd aan een betere kwaliteit van leven. Bovendien was een meer gespreide inname van eiwitten bij de hoofdmaaltijden gerelateerd aan een hogere loopsnelheid. Dit suggereert dat een gespreide eiwitinname voordelig is voor fysiek functioneren.

In **hoofdstuk 5** combineerden we deze opgedane kennis in een gerandomiseerd placebo-gecontroleerd onderzoek waarbij fysiek actieve ouderen 12 weken lang eiwitsuppletie gebruikten. Met deze eiwitsuppletie werd de gemiddelde eiwitinname verhoogd van 0.86 ± 0.23 g/kg/d naar 1.29 ± 0.28 g/kg/d en werd deze eiwitinname goed verdeeld over de dag ingenomen. We zagen een grotere stijging in het percentage vetvrije massa na het gebruiken van eiwitsupplementen vergeleken met deelnemers die placebosupplementen gebruikten.

In **hoofdstuk 6** lieten we zien dat de vitamine D status in fysiek actieve ouderen heel hoog was, zonder vitamine D supplementen te gebruiken. Dit suggereert dat ouderen met een fysiek actieve levensstijl, voornamelijk door wandelen, vaak een adequate vitamine D status hebben. Deze hoge vitamine D status is mogelijk voordelig voor de spieraanmaak.

In **hoofdstuk 7** bediscussieerden we de resultaten van de studies besproken in dit proefschrift. We concludeerden dat fysiek actieve thuiswonende ouderen een hoger percentage vetvrije massa krijgen door eiwitsupplementen te gebruiken, vooral wanneer hun gebruikelijke eiwitinname laag is. Onze resultaten ondersteunen daarmee de eiwitaanbeveling van de PROT-AGE studiegroep voor fysiek actieve thuiswonende ouderen van ≥1.2 gram eiwit per kilogram lichaamsgewicht per dag. We speculeren dat regelmatige feedback van een diëtist of een gebruikersvriendelijke applicatie én eiwitverrijkte producten in de supermarkten meer bewustzijn kan geven en mogelijk helpt in het verbeteren van de eiwitinname. Met deze verhoogde eiwitinname kunnen vitale actieve ouderen wellicht een langer, gezond en onafhankelijk leven lijden.

DATAMANAGEMENT

The data used within this thesis are collected and stored according to the Findable, Accessible, Interoperable and Reusable (FAIR) principles (1). Appropriate data management is important for 1) knowledge discovery and innovation, 2) protecting scientific integrity and 3) preservation and reuse of data sets. The raw and processed data that were generated have been stored mostly in Castoredc in which an audit trail was used to provide documentary evidence of the activities that have affected the original data. Moreover, some measurements generated automatically encoded SPSS or Microsoft Excel data files and were thus stored in here. All data files were stored at the local server of the Radboudumc, which was backed-up on daily basis to prevent data loss.

This thesis is primarily based on results of human studies, which were conducted in accordance with the principles of the Declaration of Helsinki. Additionally, a local Medical Ethics Committee approved the study protocols, including a data management plan. All subjects were well informed about the study using an information package and all subjects gave written informed consent prior to participation in the study. All study procedures were monitored by an independent researcher according to the protocol compiled by the departments of Physiology and Intensive Care of the Radboudumc. The privacy of subjects is guaranteed due to anonymization of data using a unique and untraceable individual subject code. In all data files and case report forms the individual subject code is used, which allows us to share the data if necessary. The encryption key was only available for the research team. The raw and processed data sets are stored at the department of Physiology and will be available for further analyses for at least 15 years. In order to ensure that the data is generally accessible and interoperable, all file names and data, which are used to produce the final results, were documented using applicable language for knowledge representation. Furthermore, the data generated and analyzed in this thesis is on request available from the associated corresponding authors.

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DANKWOORD

Vier jaar en vier dagen na mijn start als PhD kandidaat op de afdeling Fysiologie is het dan zo ver: de verdediging van mijn proefschrift. Het was een leerzame en leuke periode en daar wil ik graag diverse mensen voor bedanken. Aangezien het er nogal veel zijn en ik wat lang van stof ben, wil ik ook van tevoren alvast mijn excuses aanbieden voor de lengte van dit dankwoord.

Allereerst wil ik alle deelnemers van de Vierdaagse bedanken die naast het lopen van 30, 40 of 50 km ook nog de tijd en moeite namen om op ons onderzoekscentrum aan de Wedren langs te komen voor verschillende metingen. Ik heb even snel opgeteld hoeveel Vierdaagse deelnemers er in totaal hebben deelgenomen aan de onderzoeken die beschreven staan in mijn proefschrift en dat waren er maar liefst: 1.711! (Nu is deze som niet helemaal correct, want sommige deelnemers hebben verspreid over meerdere jaren meegedaan aan de onderzoeken, maar ik heb in de afgelopen jaren het koppelen van datasets als stomste taak benoemd van mijn promotietraject dus dat laat ik hiervoor graag achterwege).

In het bijzonder wil ik de deelnemers van de Prowalking studie (Vierdaagse 2017) bedanken die naast het ondergaan van een uitgebreid pakket aan metingen ook nog 12 weken lang twee keer per dag een eiwit of placebo drankje hebben genomen. Zelfs de mensen die het eigenlijk niet zo lekker vonden, hebben dit netjes gedaan. Heel erg bedankt!

Alleen kan je niks en met zijn allen kun je alles – Johan Cruijff

Deze uitgebreide onderzoeken bij dit grote aantal deelnemers was me natuurlijk nooit gelukt in mijn eentje. Ik wil dan ook graag verschillende collega's bedanken.

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vervolgens vaak binnen vijf minuten al aan de telefoon; ''ja, dan kunnen we het maar meteen ff aftikken''. Wat een luxe! Je ziet overal mogelijkheden in en met je constructieve feedback weet je onderzoeken en de bijbehorende artikelen naar een hoger niveau te tillen. Ik ben soms eigenwijs en het was wel een beetje jammer dat jij op een gegeven moment doorhad dat ik wel eens ''ja, is goed Thijs'' zei maar ''nee, ik doe het toch zoals ik zelf wil'' dacht... ☺ Binnenkort wordt jullie mooie gezin uitgebreid met een kleine. Ik wens jullie graag het allerbeste!

Aangezien het Radboudumc niet in de Gelderse Food Valley valt, heb ik ook regelmatig een beroep gedaan op mensen werkzaam in Ede en Wageningen. Allereerst, **Lisette de Groot**; hartelijk dank voor je waardevolle input op onze onderzoeksvoorstellen en de daaropvolgende artikelen. Het was erg fijn dat we op deze manier mochten genieten van jouw uitgebreide kennis over voeding en in ons geval eiwitten in relatie tot spieren.

Als het onderzoeksvoorstel vervolgens was goedgekeurd, kon ik naar je collega's van de Wageningen Universiteit: **Jeanne de Vries, Saskia Meijboom, Karin Borgonjen-van den Berg** en natuurlijk **Corine Peerenboom.** Jullie zagen de bui alweer hangen wanneer ik belde en er een Vierdaagse voor de deur stond. Dankzij jullie mooie programma's en trainingen hebben we in mijn onderzoek valide en nauwkeurige metingen kunnen doen naar de gebruikelijke voedingsinname van de deelnemers. Dank daarvoor!

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Vervolgens ga ik naar jullie buren op de campus: FrieslandCampina. **Ula Kudla**, you made sure the TKI project *Protein For Endurance* could start, which resulted in several large but thorough studies including our Four Days Marches Study: the Prowalking study. Moreover, together we started the idea for the meta-analysis. Both studies resulted in very nice publications. Thank you very much for all your efforts! Met ongelooflijk veel kennis over het onderwerp, had FrieslandCampina in **Astrid Horstman** de perfecte vervanger gevonden. Astrid, heel erg bedankt voor de fijne samenwerking, het kritisch meedenken over de resultaten en al je inhoudelijke input. Ik ben dan ook erg blij dat we onze samenwerking hebben kunnen verlengen met het Zevenheuvelenloop onderzoek. **Ellen van den Heuvel** en **Anouk Feitsma**, dankjulliewel voor jullie enthousiasme over de bot- en gewrichtmarkers. Het werkt aanstekelijk! Dankzij jullie kennis komt er een mooie publicatie uit voort!

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Dan terug naar de lieve collega's van de afdeling Fysiologie! Allereerst natuurlijk de collega's van *Room Paradise* waar ik het grootste deel van mijn PhD heb mogen doorbrengen en dan begin ik natuurlijk met mijn lieve paranimf, **Coen**! Als enige andere (huidige) dorpsbewoner zaten wij meteen op één lijn. De samenwerking rondom het Vierdaagse onderzoek verliep dan ook zeer soepel! Zonder jouw hulp, ideeën, adviezen én geruststellingen als ik stress en zorgen had over of het wel allemaal ging lukken, was het me nooit gelukt om het Prowalking onderzoek zo succesvol te laten verlopen. Ook nu je aan de andere kant van de wereld zit, ben je nog mega attent en stuur je berichtjes om me succes te wensen of te vragen of je me nog ergens mee kan helpen bij de afronding van mijn proefschrift. Op een gegeven moment moest ik zelfs even checken wat nou ook alweer het tijdsverschil was met Sydney, omdat ik vrijwel meteen reacties kreeg op mijn mailtjes en appjes. Ik ben dan ook heel blij dat je bij de promotie aan mijn zijde staat (en me zonodig kan opvangen ☺)! **Rebecca**, omdat jij je promotie combineert met werken in de kliniek, tientallen besturen en commissies met de vele bijbehorende vergaderingen en het stichten van een prachtig gezin, was jij het merendeel van de tijd maar één dag in de week aanwezig in *Room Paradise*. Dat was misschien maar beter, want met jouw aanwezigheid was het veel te gezellig om te werken! En ondanks dat we op deze dagen vooral veel bijkletsten, had je nog alles perfect op orde. Ik heb daar dan ook heel veel bewondering voor en ben blij dat ik wat heb mogen afkijken van hoe je dat allemaal doet én dat ik soms nog even voor advies bij je terecht kan. Je perfecte vervanger was je collega van de interne: **Lando**, waarmee direct de "Spreuk van de week" werd geïntroduceerd. De

spreuk van Johan Cruijff hierboven was aan het begin toen alles nog koek en ei was. Tijdens Vierdaagse periodes moesten we met zwaarder geschut komen. Toen je tig telefoontjes kreeg van deelnemers aan het onderzoek en ik nooit op mijn plek zat en jij dus maar op nam met *''de secretaresse van Dominique''*, kwam de spreuk: *''Je hoeft niet gek te zijn om hier te werken, maar het maakt het wel een stuk makkelijker''*. Wanneer ik je heel vaak had ingedeeld om bloed te prikken, kwam de spreuk: *''Het mooiste werk is samenwerk''*. En toen niet alleen je schaar, nietmachine, prullenbak maar ook de weegschaal werd gejat, kwam de spreuk: *''Een blije collega is het halve werk''* om de boel een beetje te sussen. Ik betwijfel of de spreuken van Coen en mij hebben geholpen, want zelf schreef je: *''Two things are infinite. The universe and human stupidity. .... And I'm not sure about the universe"* - Albert Einstein. Gelukkig hebben we het in de andere weken van het jaar best wel gezellig en ik kan nog steeds lachen als we weer eens zo erg kibbelen dat het net lijkt alsof wij een getrouwd stel zijn (en dus niet jij en Eline), waarop jij de al 10 minuten durende discussie beëindigt met *''Maar goeddd, ik zeg maar zoo, ik zeg maar niks''*, die onze nieuwe roomie **Cindy** aanvulde met *''zoo is tenslotte korter dan dierentuin''*. Deze droge humor van ons-Cin was dan ook de reden waarom wij je meteen met luid gejuich en applaus zijn gaan ontvangen als je arriveert in *Room Paradise*. Eerlijk is eerlijk, van tevoren had ik wel mijn bedenkingen over onze #fitgirl of zoals je zelf zegt #haverkut, maar boy, was I wrong! Je bent super lief en gezellig en ik heb ontzettend veel bewondering gekregen voor jouw sportiviteit, harde werk en doorzettingsvermogen. Elke ochtend kom je weer met 3 hutkoffers aan, zodat je na je fulltime promotietraject die je uitvoert op de HAN, Maastricht Universiteit én Radboudumc, nog even sporters diëtistisch advies kan geven, de Gemert Cityrun organiseert om tot slot zelf nog even te hardlopen, spinnen, krachttrainen of te wielrennen. Kortom, respect! Aangezien *Room Paradise* tegenwoordig op de grootste kamer van de afdeling zit, was er plek voor onze nieuwe aanwinst: **Thijs**. En aangezien deze arme jongen de derde Thijs van de afdeling was, werd je al snel Sparkle (naar zijn achternaam Vonk) genoemd. Excuus dat ik zo gretig gebruik heb gemaakt van deze bijnaam... Daarnaast maakte je al in week 1 de grote fout om te laten merken dat je computerkennis hebt. Dat heb je geweten met mij tegenover je, aangezien ik die week erna mijn laptop heb laten crashen met alle gevolgen van dien. De adviezen van Lando om mij te negeren als ik weer eens zit te vloeken op mijn laptop (''aangezien ze vanzelf wel weer ophoudt en/of naar Martijn of Hugo rent'') waren dan ook tevergeefs. Toch ben je nog niet gevlucht en zijn we erg blij met onze nieuwe, gezellige, vrolijke collega die gelukkig ook onze memes, muziek en humor kan waarderen.

Na lang eenzaam bovenaan te hebben gestaan met *Room Paradise* voor de titel ''Leukste kamer van de afdeling'', was daar opeens *Room Mancave*. Een geduchte tegenstander. *Room Mancave* werd mooi ingericht met een relax fauteuil en we hoorden regelmatig hard gelach vanuit deze hoek. Maar toen het Mancave magazine in onze mailbox verscheen, wisten we zeker dat *we needed to step up our game*. Door schrijftalent **Malou** te scouten als Queen of the Mancave, worden wij elke maand verblijd met een hilarisch, goedgeschreven update over alle

ins en outs van de mannen op deze kamer: Thijs, Bram en Geert. Doordat ik het geluk had dat Malou eerst bij mij stage heeft gelopen, wist ik al lang hoe goed jij in je werk bent. Hier wordt dan ook veelvuldig gebruik van gemaakt door de mannen. Hulp bij SPSS, PowerPoint, SNAP, Castor en liefdeslevens. Niks is jou te veel. Ik weet dan ook zeker dat jij een zeer succesvol promotietraject in gaat! **Thijs**, jij hebt al verschillende successen geboekt door Malou's hulp aan te nemen. Maar ik zal niet onderkennen wat je zelf allemaal in je mars hebt. Je hebt ontzettend veel kennis waarmee je een mooi onderzoek bent gestart. En met je lieve, geduldige karakter weet je ook nog eens ontzettend goed jouw kennis over te brengen aan je collega's die iets minder verstand hebben van sommige zaken. Erg knap! Ik blijf je gezellig aanmoedigen als ik je weer met een witte jas weg zie steppen! **Bram**, ook bij een offday word ik spontaan vrolijk als jij ergens enthousiast over bent: ''geweeeldig, dat is toch mooi Dominique? Echt prachtig''. Met ditzelfde enthousiasme ben je bezig met jouw project en dat wordt daarom sowieso een groot succes! **Geert**, de best geklede man van de afdeling en toch ook wel het luxe paardje van de afdeling. Je hebt het analyseren van de vele echo's even tijdelijk ingeruild voor een leuk en interessant onderzoek in Liverpool. Natuurlijk heb je er ook daar weer voor gezorgd dat je een prachtig appartement hebt met de meeste fantastische voorzieningen. Petje af! In de tussentijd wordt jouw plekje in gebruik genomen door **Jenske**. Hartstikke leuk dat jij onze afdeling bent komen versterken! Je bent recent gestart met een relevant project waarmee je de verbinding maakt tussen Rijnstaete en het Radboudumc. Hartstikke knap! Veel succes gewenst!

Dan naar de kamer waar er iets geconcentreerder wordt gewerkt, met wellicht als gevolg dat deze kamer de titel *Room High Impact* kreeg (maar aangezien jullie dit zelf zo hebben genoemd, hebben sommige collega's (of was ik het toch zelf?) daar *Room Socially Incapable* van gemaakt). Nee zonder geintjes, het is meer dan terecht dat jullie harde werk wordt beloond met mooie publicaties en daar mogen jullie trots op zijn! **Hugo**, ik heb het al vaker gezegd maar ik zeg het nog een keer zwart op wit: je bent té goed voor deze wereld! Dankjewel voor al je belangeloze hulp bij de Vierdaagse, je goede inhoudelijke inzichten én je computer hulp! Want als ik weer eens over de gang riep: *''Huuuuugooooooooo....''*, wist jij al hoe laat het was en zette je direct alles aan de kant om mij te helpen. Dank! **Yvonne**, hartstikke knap hoe jij deze ontzettend grote studie (bijna) hebt afgerond! We begonnen ongeveer tegelijk aan ieder ons eigen grote project waardoor we vaak pas gezellig konden kletsen als het al zeer stil was op de afdeling en dus eigenlijk al te laat en onze vriendjes al lang op ons zaten te wachten met het eten. Sorry, dat mijn project maar 13 weken duurde en ik er daarna niet meer zo vaak was om jou gezelschap te houden! Tot slot, de meest hardwerkende collega van de afdeling: **Vincent**! We wisten het al langer maar bij de afronding van je PhD heb je nog maar even dubbel en dwars aangetoond wat een bikkel jij bent! Pauzes zijn volgens jou overrated, maar gelukkig kan ik wel altijd op je rekenen voor onze lunchwandeling. Maar toen ik onlangs al de hele dag in Papendal was en jij me appte wanneer we gingen wandelen en je dus blijkbaar nog niet eens naar het koffieapparaat was gelopen, gingen er bij mij toch wel wat alarmbellen af ☺ Je nam mijn

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Esmee, door jouw uitgebreide statistische en epidemiologische kennis ben je vaak meer bezig met het helpen van je collega's, dan met je eigen project. Onze excuses daarvoor én dankjewel natuurlijk! Ik verheug me nu al op je inaugurale rede wanneer je wordt benoemd tot professor! In de tussentijd zal ik geen prullenbakken meer van je kamer lenen. **Eline**, toen jij en Lando stage kwamen lopen wist ik vrijwel meteen dat ik geen werk aan jullie zou hebben. Jullie hadden alles tot in de puntjes geregeld, wat Maria ook direct had gezien. Dus toen jij de beurs won om een PhD te gaan starten, mocht je meteen een ontzettend groot onderzoek gaan uitvoeren. Niet iedereen heeft dat in zich, maar jij hebt het fantastisch gedaan. Ik weet zeker dat je over een paar jaar een super goede huisarts zult zijn (maar eigenlijk ben je dat nu al), met een prachtig proefschrift op zak! **Lisa**, leuk dat er met jouw komst meer voedingsonderzoeken worden gedaan op onze afdeling. Je doet dit met verve en ik ben benieuwd naar de uitkomsten! **Yannick**, gelukkig mag ik je soms gezellig komen helpen op Papendal, anders zagen we je bijna niet! Je hebt het Thermo Tokyo project zo goed georganiseerd dat er ontzettend veel sporters langs willen komen voor metingen. Super leuk dat je dit project gaat voorzetten in een promotietraject! **Carlijn**, je bent er maar 1 dag in de week maar je hoort er zo bij dat ik soms bijna vergeet dat je er weinig bent. Super dat we altijd op je kunnen rekenen bij onze uitjes. **Virginia** and **Daria**, it is nice to have you guys at our department. I can use the English refresher course ☺ I am curious for your studies which are about to start!

Bregina, ik bedenk me vaak of ik jouw lach al heb gehoord om te beoordelen of jij aanwezig bent. Je kritische doch rechtvaardige opmerkingen zorgen ervoor dat wij als onderzoekers kritisch blijven kijken naar onze onderzoeken waarmee we het naar een hoger niveau kunnen brengen. Daarnaast is je hulp bij de onderzoeken én je gezelligheid in de pauzes van onschatbare waarde. Heel erg bedankt voor het maken van mijn prachtige kaft! Van Bregina is het natuurlijk de meest logische overgang om naar **Joep** te gaan. Joep, je bent een ontzettend gezellige collega die zijn zaakjes goed op orde heeft. Mijn complimenten! Ik vind het leuk dat we samen kunnen werken! **Silvie**, ik heb ontzettend veel bewondering voor jouw kennisniveau en gedegen manier van onderzoek doen. Ontzettend knap hoe je dat allemaal combineert met je jonge, knappe gezin! **Paul**, ik denk niet dat we ooit zo blij zijn geweest met de komst van een nieuwe collega ☺ Ik heb al mogen zien hoe goed jij bent in het geven van onderwijs en ik ben ervan overtuigd dat het MT geen betere keuze had kunnen maken! **Dick**, ik vind het nog steeds bewonderenswaardig dat jij zelfs om 01.00 uur 's nachts goede, scherpe, behulpzame feedback

kan geven op de artikelen van de vele PhD'ers die jij begeleidt. En dat terwijl je het allemaal combineert met een gezin met 3 kinderen. Binnenkort gaan jullie verhuizen naar een prachtig huis. Hopelijk heb je dan ook wat meer tijd om er goed van te genieten! **Pascale**, dankjewel voor je goede hulp en gezellige kletsbezoekjes!

Tot slot, wil ik graag vier gezellige, behulpzame ex-collega's bedanken voor de leuke tijd die we samen hebben doorgebracht. **Martijn** en **Anke**, dankjulliewel voor het warme welkom in *Room Awesome*. Jullie hebben me goed op weg geholpen en gezorgd voor een hoop gezelligheid! **Nathalie** en **Thessa**, onze gezamenlijke tijd op de afdeling Fysiologie was kort maar krachtig. Jullie zijn toppers en ik ben blij dat we even gezellig collega's zijn geweest!

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Tijdens mijn promotie heb ik naast Lando, Eline en Malou nog meer studenten mogen begeleiden: **Hannah, Carlijn, Carol, Floor, Amy** en **Danielle**! Bedankt voor jullie ontzettend waardevolle bijdrage aan de verschillende onderzoeken. Daarnaast had ik ook nog eens de luxe dat veel studenten Voeding & Diëtetiek mij hebben geholpen met het afnemen van de voedingsdagboekjes van alle deelnemers. **Vienne, Merel, Lizet, Janneke, Ivy, Danique, Kevin, Sofie, Stefanie, Wieta, Lieke KG, Lieke vL, Anna, Mirna, Maxime, Fleur, Joosje, Juliette, Emily, Noortje, Charlotte, Emma** en **Michelle**! Heel erg bedankt en ik wens jullie allemaal veel succes met jullie toekomstige carrières!

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soms ook wat er op het eind van de avond is gebeurd). De **maatjes + aanhang** (het wordt hoog tijd voor een betere naam), dankjulliewel voor de gezelligheid en leuke weekendjes weg. Lieve **WUR-meiden**: **Manon, Charlotte, Anita, Bianca** en **Sylvie**, **boardgame compliance** ex-collega's **Tim, Wesselien** en **Denise** en natuurlijk **Linda**, we zien elkaar eigenlijk te weinig, maar als we elkaar zien is het meteen weer als vanouds en mega gezellig! Ik hoop dan ook dat er nog vele etentjes zullen volgen! Tot slot, lieve **Ilse** en **Vera**, we korfballen al tijden niet meer samen maar dat heeft niks aan onze band veranderd. Dankjulliewel voor jullie leuke, lieve en gezellige karakters!

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Lieve **ooms, tantes, neven** en **nichten**. Ik ben trots op onze gezellige en liefdevolle familie en dat wij elkaar nog zo veel zien ondanks dat opa en oma kippen en opa en oma Pukkie helaas al lange tijd niet meer onder ons zijn. Oma Lieske kon mij als geen ander laten zien hoe waardevol het is om vitaal oud te worden en dat was dan ook een grote inspiratiebron voor dit proefschrift om hopelijk een steentje bij te dragen aan dat alle opa's en oma's op zo'n manier oud mogen worden.

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Papa en **mama**, jullie zijn de liefste ouders die ik me maar kan wensen. Jullie staan altijd voor me klaar en hebben het beste met me voor. Jullie hebben me geleerd zelfvertrouwen te hebben, mensen respectvol te bejegenen, hard te werken, maar ook te genieten van het leven. En dit is maar een kleine greep uit de normen en waarden die ik van jullie heb mogen leren die me de afgelopen jaren ontzettend goed van pas kwamen om dit proefschrift goed af te ronden. Dankjulliewel en geniet van de verdiende rustigere tijden die in het verschiet liggen! **Jean-Paul** en **Daphne**, ik had me geen lievere broer en zus kunnen wensen. Als jongste heb ik me altijd beschermd gevoeld door jullie en hebben we het vooral heel gezellig met elkaar. JP, dankjewel dat je bij de familie etentjes mijn vele verhalen geduldig aanhoort en Daphne, dankjewel dat

je zo hard lacht op mijn grapjes! ☺ Als iemand vele verhalen van mij heeft aan moeten horen ben jij het wel zus, want elke terugrit naar huis had ik jou weer aan de lijn om de dag door te nemen. Het was dan ook niet meer dan logisch dat jij mijn paranimf zou zijn, want jij weet het allerbeste wat er elke dag heeft gespeeld. Je steunde me, gaf me adviezen, vroeg me hoe een belangrijk overleg was gegaan en liet blijken dat je trots op me bent. Maar lieve zus, wat ben ik trots op jou! Je werkt ontzettend hard, bent mega attent, hebt een prachtig huis en samen met je lieve man **Stijn** hebben jullie een prachtige zoon op de wereld gebracht. Jullie zijn fantastische ouders en ik ben heel trots dat ik de peettante van Silvijn mag zijn!

Lieve **Daan**, bijna 10 jaar geleden leerden we elkaar kennen en wist ik meteen dat het goed zat. Je hebt misschien wel de belangrijkste taak verzorgd binnen dit promotietraject. Jij zorgde voor een liefdevol, relaxed en gezellig thuis, waardoor ik na al die tijd nog steeds regelmatig met tegenzin wegrijd van huis 's ochtends omdat ik jou niet wil missen. Vervolgens liet ik dan op de werkvloer regelmatig jouw naam vallen met als gevolg dat mijn collega's behoorlijk wat weten over jou. Sorry, maar waar het hart vol van is, daar loopt de mond van over! Het was voor jou al een drukke tijd, maar er komt ook nog een spannende tijd aan met de nieuwe fabriek van jullie eiwitrijke product: kaas. Ik ben enorm trots op hoe hard je je daarvoor inzet en ik hoop dat ik jou ook wat welverdiende rust kan geven thuis. Hopelijk komen er daarna weer rustigere tijden aan en krijgen we lekker veel tijd die we samen door kunnen brengen. En anders wacht ik geduldig wat langer, want allerliefste, ik heb heel veel zin om samen met jou vitale, actieve oudjes te worden!

LIST OF PUBLICATIONS

ten Haaf D.S.M., Eijsvogels T.M.H., Bongers C.W.G., Timmers S., de Groot L.C.P.G.M., Hopman M.T.E. Protein supplementation improves lean body mass in physically active elderly: a randomized double-blind placebo-controlled trial. Journal of Cachexia, Sarcopenia and Muscle, 2019. [Epub ahead of print]

ten Haaf D.S.M., Balvers M.G.J., Timmers S., Eijsvogels T.M.H., Hopman M.T.E., Klein Gunnewiek J.M.T. Determinants of vitamin D status in physically active elderly in the Netherlands. European Journal of Nutrition, 2018. [Epub ahead of print]

ten Haaf D.S.M., Nuijten M.A.H., Maessen M.F.H., Horstman A.M.H., Eijsvogels T.M.H., Hopman M.T.E. Effects of protein supplementation on lean body mass, muscle strength and physical performance in non-frail, community-dwelling elderly: a systematic review and meta-analysis. American Journal of Clinical Nutrition, 2018; 108(5):1043-59.

Terink R., **ten Haaf D.S.M.**, Bongers C.C.W.G., Balvers M.G.J., Witkamp R.F., Mensink M., Eijsvogels T.M.H., Klein Gunnewiek J.M.T., Hopman M.T.E. Changes in iron metabolism during prolonged repeated walking exercise in middle aged men and women. European Journal of Applied Physiology, 2018; 118: 2349.

ten Haaf D.S.M., van Dongen E.J.I., Nuijten M.A.H., Eijsvogels T.M.H., de Groot L.C.P.G.M., Hopman M.T.E. Protein Intake and Distribution in Relation to Physical Functioning and Quality of Life in Community-Dwelling Elderly People: Acknowledging the Role of Physical Activity. Nutrients. 2018;10(4).

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Janssen L., Allard N.A.E., **ten Haaf D.S.M.**, van Romburgh C.P.P., Eijsvogels T.M.H. Hopman M.T.E. First-Aid Treatment for Friction Blisters: "Walking Into the Right Direction?". Clinical Journal of Sports Medicine, 2018;28(1):37-42.

van der Worp M.P., **ten Haaf D.S.M.**, van Cingel R., de Wijer A., Nijhuis-van der Sanden M.W., Staal J.B.. Injuries in runners; a systematic review on risk factors and sex differences. PLoS One. 2015;10(2):e0114937.

ten Haaf D.S.M., van der Worp M.P., Groenewoud H.M., Leij-Halfwerk S., Nijhuis-van der Sanden M.W., Verbeek A.L., et al. Nutritional indicators for gastrointestinal symptoms in female runners: the 'Marikenloop study'. BMJ Open. 2014;4(8):e005780.

CURRICULUM VITAE

Dominique ten Haaf werd op 1 mei 1990 geboren te Ottersum. Na haar middelbare school periode aan het Elzendaalcollege in Boxmeer, startte ze in 2007 met de opleiding Voeding & Diëtetiek aan de Hogeschool Arnhem Nijmegen te Nijmegen. Daar behaalde ze in 2011 haar diploma om vervolgens de Master Nutrition & Health aan de Wageningen Universiteit te gaan volgen. Met een 9.0 voor haar afstudeerstage bij de afdeling IQ Healthcare van het Radboudumc, rondde ze in 2013 deze opleiding succesvol af. Binnen haar afstudeerstage waar ze onderzoek deed bij de Marikenloop, kwam ze voor het eerst in aanraking met Prof. Maria Hopman. Na een tijdje als labelling specialist te hebben gewerkt voor KTBA People in Food, startte Dominique in 2015 met haar promotietraject op de afdeling Fysiologie van het Radboudumc. Onder leiding van Prof. Maria Hopman en Dr. Thijs Eijsvogels ondervonden ze dat de eiwitinname in de fysiek actieve oudere groep van Vierdaagse lopers vaak te laag was. Naar aanleiding daarvan zijn diverse vervolgstudies opgericht om de gevolgen hiervan te onderzoeken, om uiteindelijk een grote interventie studie op te richten waarbij het effect van eiwit suppletie op spiermassa, kracht en fysiek functioneren werd onderzocht binnen fysiek actieve ouderen. Tijdens haar periode als promovendus presenteerde zij haar bevindingen op verschillende nationale en internationale bijeenkomsten in de vorm van posters en mondelinge presentaties. Daarnaast heeft ze meerdere masterstudenten van de opleidingen Geneeskunde, Biomedische Wetenschappen (Radboud Universiteit), Evidence Based Practice in Health Care (Universiteit van Amsterdam), Nutrition & Health (Wageningen Universiteit) en Human Movement Sciences (Maastricht Universiteit) begeleid en coördineerde Dominique onderzoeken bij de Nijmegen Exercise Study en de Nijmeegse Vierdaagse. Momenteel is ze werkzaam als postdoc onderzoeker bij de afdeling Fysiologie, waar ze werkt aan diverse onderzoeksprojecten. Zo heeft ze reeds een onderzoek gedaan bij de Zevenheuvelenloop 2018 naar het effect van eiwitsuppletie op spierpijn en spierschade na een 15 km hardloopwedstrijd.

Institute for Health Sciences
Radboudumc

RIHS PORTFOLIO

Name PhD candidate: **D.S.M. ten Haaf**	**PhD period: 01-04-2015 – 30-09-2018**
Department: **Physiology**	***Promotor(s):*** **Prof. M.T.E. Hopman**
Graduate School: **Radboud Institute for Health Sciences**	***Co-promotor(s):*** **Dr T.M.H. Eijsvogels**

	Year(s)	ECTS
TRAINING ACTIVITIES		
a) Courses & Workshops	e.g.	e.g.
- Mindfulness	2018	3.0
- Scientific Integrity	2016	1.0
- Management voor promovendi	2016	2.0
- Effectieve schrijfstrategieën	2015	3.0
- Junior refereren ¨Introduction to meta-analysis¨	2015	3.0
- BROK (basic course regulations and organization for clinical researchers	2015	1.5
- Radboud Institute for Health Sciences Introduction course for PhD students	2015	0.5
- Introduction day Radboudumc	2015	0.5
a) Seminars & lectures		
- Radboud Research Rounds	2015-2016	0.5
- Prof. James Skinner (Lecture: The influence of genetic factors on training and health)	2017	0.1
- Prof. Duck-Chul Lee (Lecture: Health benefits of physical activity & fitness: what type of physical activity is best for health?)	2017	0.1
- Prof. Bo Fernhall (Lecture: Inflammation, exercise and arterial function)	2017	0.1
a) Symposia & congresses		
- Food Valley Summit, Ede (oral)	2018	1.0
- International Congres of Nutrition, Buenos Aires, Argentinië (poster)	2017	1.3
- International Sports + Exercise Nutrition Conference, Newcastle, Engeland (oral)	2015	1.3
- Nutritional Science day, Heeze (oral)	2015	1.0
a) Other	e.g.	
- Vascular damage theme meetings	2015-2018	0.5
TEACHING ACTIVITIES		
a) Lecturing		
- Course: Belasting en belastbaarheid; practica en werkgroepen	2015-2018	1.0
- Student education at the department of Physiology	2016-2017	0.2
- Minor Moving questions: practica en werkgroepen	2017-2018	0.5
- Meet your PhD (mentor van 6 eerstejaars studenten Biomedische wetenschappen)	2016-2018	0.5
a) Supervision of internships / other		
- Lando Janssen, master Geneeskunde (First aid treatment for friction blisters: "walking into the right direction?")	2015	1.0
- Eline Allard, master Geneeskunde (Risk factors for blister formation and delayed blister healing)	2015	1.0
- Hannah Nuninga, master Geneeskunde (Quantification of change in quadriceps muscle mass and strength during 4 weeks exercise and protein supplementation)	2016	1.0
- Carlijn van der Wielen, master Geneeskunde (The impact of prolonged moderate-intensity walking exercise on iron metabolism)	2016	1.0
- Carol van der Kust, master Evidence Based Practice in Health Care (Associations between protein intake and protein distribution over the day on fat free mass and handgrip strength in active older adults)	2016	1.5
- Malou Nuijten, master Biomedische Wetenschappen (Protein intake in physically active elderly)	2016	1.5
- Amy van den Tillaer, master Nutrition & Health (The influence of endurance exercise on lean body mass, leg muscle strength and contractile function in vital active elderly)	2017	1.5
- Danielle Stolzenbach, master Geneeskunde (Relation of endurance training and physical performance in physically active elderly)	2017	1.5
- Floor Fransen, master Human Movement Sciences (The effect of protein supplementation on strength and contractile properties of knee extensor muscles in physically active older individuals)	2017	1.5
TOTAL		**34.1**

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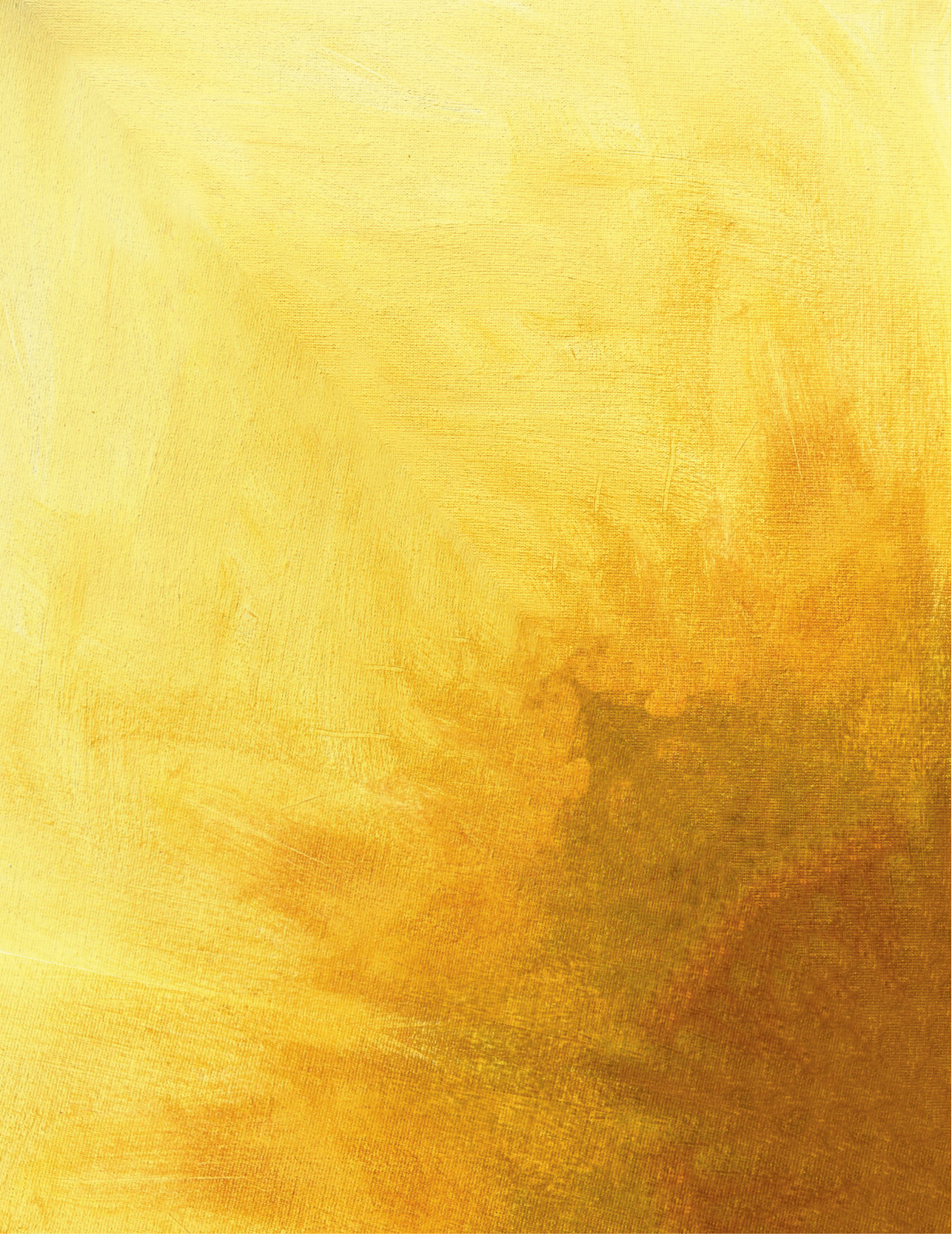

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